On the Relation between the Structure and the Development of the Centrifuged Egg of the Frog.

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Contents.

I. INTRODUCTORY.

It is to the structure and constitution of the germ-plasm, of the cytoplasm as well as of the nucleus of the germ-cell, that the path of inquiry into the internal causes of the development

of the organism, the causes which determine in each generation the reproduction of the specific form, inevitably leads back. It is not a little curious that a method of experimental research which has already given something and promises perhaps to contribute still more to the analysis of the factors resident in the egg-cytoplasm should owe its existence to those classical experiments of the physiologist Emil Pflüger, which led their author to formulate the doctrine of the "isotropy" of the ovum, and to deny to the egg-cytoplasm any structure, or at least any significant structure at all.

Normally, the eggs of the frog and toad—Pflüger used the ova of Bombinator—are not free to rotate inside their tightly adherent jelly-membranes when first they are laid. But shortly after and as a consequence of insemination there is developed between the egg and the innermost layer of the jelly a perivitelline space, inside which the egg then rotates until its axis becomes vertical with the heavier yolk-pole below. It will be recalled that Pflüger succeeding in inhibiting the formation of this space, though not the subsequent segmentation and development, by giving each egg only a minimal drop of sperm-containing water. Such eggs could be kept forcibly inverted in any position in which the experimenter chose to place them, with the original axis making any angle with the vertical, and, except when the inversion was absolutely complete, were capable of normal segmentation and development. In cleavage it was found by Pflüger that the first two divisions were vertical and the third horizontal, just as in the undisturbed egg the first two (meridional) and third (latitudinal) are also respectively vertical and horizontal, while later on the dorsal lip of the blastopore appeared just below the actual equator, and travelled down to the actual lower pole in the plane including the actual (vertical) and the original (inclined) egg-axes. The conclusion drawn from these facts—that the embryo may be developed from any part of the egg, that the egg is consequently isotropic, and only undergoes a specific development because it is always brought under the same external conditions—was not allowed to remain unchallenged for long.

First Roux showed that the constant directive influence of gravity might be easily eliminated by keeping the eggs in a perpetual state of slow rotation, under which circumstances their segmentation and development bear the same relation to the original axis and polar structure as is ordinarily the case. And secondly, Born's examination of sections showed at once that in these forcibly inverted eggs there takes place a redistribution of material, the lighter liquid plasma ascending, the heavier yolk-particles descending until there is conferred upon the ovum a new structure with a new axis, which is of course vertical, and has unlike animal or protoplasmic and vegetative or yolk poles. The pigment, however, is not wholly shifted, though some is carried up into the lighter plasma. The upturned white pole remains white, or at most becomes greyish. To this new structure—which may have any relation, make any angle with the original structure—the cleavage and development of the egg has the same relation as normally. With regard to the new axis the first and second furrows are meridional, the third latitudinal, while the head of the embryo appears near the new animal pole. The median plane of the embryo is that plane in which both the original and the new axes lie, for the streaming up and down of the plasma and yolk take place symmetrically about that plane, and the bilaterality so conferred upon the egg-contents persists as that of the embryo.

The very experiments, therefore, which were vainly imagined to prove the isotropy of the cytoplasm have only succeeded in emphasising the significance of the structure of the ovum for the development of the embryo.

This experiment can be performed in what is, I think, a more convenient manner by substituting for gravity a centrifugal force, greater than gravity, but not too large. As soon as the eggs have been inseminated they are placed with sufficient water to allow the jelly to swell and the perivitelline fluid to be exuded, in the tube of the centrifuge, and centrifuged at once for a short time. The eggs, of course, lie haphazard in the tube, with their axes making any angle with the

direction of the force, and since they cannot move until the perivitelline space has been developed their contents are re-distributed as in Pflüger's experiment. They are then removed from the machine. It will be found that they do not turn over, that the first and second cleavages are parallel, the third at right angles to the direction of the force, and that the dorsal lip of the blastopore appears just on the centrifugal side of the (new) equator of the egg.

Wetzel and Hertwig have recently employed this method in the study of the particular case of complete inversion, which does not apparently prevent the formation of the embryo, as stated by Pflüger.

The credit of subjecting the eggs of the frog to a still higher centrifugal force belongs to O. Hertwig. He found that the segmentation of such eggs was meroblastic. A cap of small cells or blastoderm was formed resting upon an undivided though nucleated yolk, and these yolk-nuclei were large and irregular, resembling the giant nuclei found in the large-yolked eggs of fishes and other forms. If removed from the centrifuge in time these eggs developed, though monstrosities (spina bifida) were frequent.

More recently Morgan, Konopacka and McClendon have all made contributions to the problem of the relation between this disturbance of the egg-structure and the development of the embryo.

Morgan has shown that with a still higher speed (1600 revolutions per minute for seven miuntes, R = 5 in., f. = 370 g.) the egg of the American species Rana sylvatica develops a grey patch round the animal pole owing to the heavy pigment being driven centrifugally into the interior of the egg like a plate. In these eggs the first and second furrows are approximately meridional, but those of the third phase are abnormal in being again meridional. The dorsal lip of the blastopore is in the normal position, but the yolk plug may be pigmented. The tadpole is antero-ventrally unpigmented, but this defect may be made good in later stages. Morgan refers to but does not follow up nor adequately describe

an interesting abnormality which he says is not uncommon. In this the front end of the nervous system is malformed, the neural folds each terminating in a knobbed extremity. With still longer exposures the yolk fails to segment, but "the more fundamental questions relating to the distribution of the materials of the egg, and the interpretation as to whether these visible substances are organ-forming or organ-determining have not been discussed. It is evident that the black pigment has no such function, but further experiments will be necessary in order to determine what value the other substances in the egg may have"—a conclusion with which we may cordially agree.

The principal object of the work of Konopacka (on Rana fusca) is to discover whether there is any alteration in the sensitiveness of the egg to this disturbing agent during the early stages of fertilisation and segmentation, and it does indeed appear that the number of abnormal embryos is greater when the eggs are centrifuged during the early cleavages than when the operation is performed upon unfertilised intra-uterine eggs, or on eggs in process of fertilisation. Apart from this, however, the paper contains a description and figure, the first published, of the alteration in the structure of the egg-cytoplasm, as well as an account of some of the monstrosities produced. With short exposures to a fairly high acceleration (f = 228 g.) the grey area of Morgan appears, and is seen in sections as a layer of yellow vacuolated hyaloplasm surmounting the inwardly driven pigment and the yolk. With longer exposures a white hyaloplasmatic layer is interpolated between the vacuolated and pigmented layers, while another vacuolated layer appears between the white layer of hyaloplasm and the yolk while the first vacuolated layer becomes folded. No attempt is made, however, to investigate the gradual genesis of these layers, nor to ascertain the chemical nature of the substances composing them. Amongst the abnormalities described are numerous half-embryos, a portion of the egg having remained unsegmented, embryos with persistent blastopores, and tailed but headless monsters.

The last, as I hope to show, are of particular interest, but the author figures only the external appearance of one, whose development has been very much arrested, and gives no detailed description of the anatomy of either this or other stages of the malformation.

The most recent work of all, that of McClendon, marks a very great advance, for here we have for the first time a chemical analysis of the various layers into which the egg cytoplasm is separated by the centrifuge. By the use of a considerable force for a short time (f = 2771 g., for five minutes) the eggs of Rana pipiens and Acris gryllus become divided into zones. In the first there are three zones, A, a yellow centripetal cap (corresponding to the grey area of Morgan and the vacuolated hyaloplasm of Konopacka) which consists of globules—soluble in oils and ether—which are mostly fat but partly lecithin, inasmuch as there is some phosphorus in the alcohol extract of this layer. The water content of this layer is 50 per cent. The second layer, B, is grey, of translucent protoplasm, 82 per cent. of which is water; there is also some fat and lecithin. The third layer, C, is black, it consists of the yolk and pigment, contains 42 per cent. of water, some fat, a good deal of lecithin, and a large quantity of protein which is rich in phosphorus and therefore supposed to be a nucleo-protein. In Acris the first layer consists of a yellow cap and a white ring, while the pigmented portion of the third is divided into three rings.

It goes without saying that the chemical composition of the several layers could not have been ascertained from any investigation of individual eggs. For this purpose a whole mass of eggs was centrifuged, the layers separated and analysed. The method was to take, not, of course, the laid egg with its coating of jelly, but ripe ovarian eggs. In actual practice the whole ovary was removed, including not only the fully grown ova, but all the young ones as well, washed and squeezed through bolting cloth to get rid of the stroma. The pulp so obtained was then centrifuged. This appears to me to introduce a certain error. I must also point

out that McClendon has determined only the phosphorus content of the ethereal and alcoholic extracts and of the solid protein-containing residues, and has given no further proof of the presence of lecithin and nucleo-protein. These points, therefore, and of course many others remain for investigation, but McClendon's work is certainly a beginning, and the priority belongs to him.

I myself had frequently had occasion to examine, somewhat cursorily, the development of the centrifuged eggs of the frog, and it had occurred to me quite independently, as indeed it would naturally occur to anyone with such eggs under his eyes, and before I became acquainted with McClendon's papers, that the layers might be obtained in sufficient quantity for chemical investigation if a mass of egg-pulp were centrifuged. Moreover, if the abnormal development of the embryo really is a consequence of the derangement of the materials of the cytoplasm, it ought to be possible to relate a certain degree of malformation with a certain degree of derangement by comparing on the one hand the composition of the several layers in masses of egg-pulp centrifuged at different speeds, and on the other the development of embryos from eggs centrifuged at corresponding speeds. Such an investigation, though it would not certainly lead to great results, yet seemed well worth undertaking. But before that could even be possible there was a preliminary question to answer. As a result of similar experiments on the ova of various Invertebrates it has been seriously suggested that the polarity of the egg, to which the structure of the embryo bears such a very definite relation, is not determined by the disposition of the various visible and separable constituents of the cytoplasm, which, indeed, so it is maintained, may be driven by the centrifuge to any region of the egg, leaving the original polarity intact, and without prejudice to the normality of development.

It became necessary, therefore, first of all to inquire into the structure of the embryos produced from such ova, and the relation of that to the derangement of the egg materials. For only if it should turn out that the polar structure of the egg

to which the normal development of the embryo is related is determined, in part at least, by a certain arrangement of the visible materials which can be actually separated by the centrifuge, would any attempt to ascertain their chemical nature be of the slightest value, however successful. It was with these objects in view that the experiments which are now to be described were undertaken in March and April last.

I shall deal first with the structure and development of the centrifuged eggs (which in any case have not yet been completely elucidated), and afterwards give such observations as I have made on the chemical composition of the various constituents of the cytoplasm.

A general discussion of these results and of their bearing on the large problem of the nature and origin of the polarity of the ovum and its relation to the development of the embryo will be found in a final chapter.

I will only add here that I am under very great obligations to my friend Dr. Ramsden, not only for permitting me to work in the Laboratory of Physiological Chemistry, but also for giving me the most valuable advice and much personal assistance.

I am also very much indebted to Professor Dreyer for allowing me the use of the centrifuge in his laboratory, and to Dr. Scott for the loan of a freezing microtome.

II. THE EXPERIMENTS.

(A) The Structure and Development of the Centrifuged Egg of the Frog.

(1) Methods.—I have used the eggs of the common English frog. The eggs were taken from the uterus, inseminated, and allowed to remain in water for about an hour until the jelly had swollen and the perivitelline fluid been exuded. The eggs turned into the normal position with the axis vertical and the white pole below. They were then

placed on the centrifuge, which was at first set in gentle motion to turn the axes of the eggs into the direction of the force, and then more rapidly to bring about the desired effect. The eggs were thus centrifuged in the direction of their axes with the animal pole centripetal.

Various speeds were used, but I am unable to state in any case the precise number of revolutions per minute.

In series G the electrically driven machine was used, and at a speed of about 1500 revolutions. In the others a water-driven machine was employed, at speeds varying from about 1100 to 3000 revolutions a minute. These speeds, which I call I, II, III and IV, I being the lowest, were determined by the angle through which the tap of the apparatus was turned, Owing, however, to the inconstancy of the water-pressure they probably varied in the course of the experiments.

The radius in both machines was about three inches.

Different exposures were used, from five to thirty minutes.

After the treatment the ova were removed to vessels of clean water.

(2) Details of the Experiments.

G.

Centrifuged 27 : iii : '13 on the electrically driven machine at bottom speed.

1. One hour after insemination, centrifuged for 5 minutes.

A grey patch appears round the animal pole.

The first two segmentation furrows are normal.

28 : iii : '13.—The eggs have segmented normally.

The grey patch is no longer visible.

8 : iv : '13.—The tadpoles have hatched out, but some are abnormal, the yolk-sac being swollen, and are inert.

All preserved in picric acid.

Of these tadpoles 41 are apparently normal, i.e. like the controls, 12 abnormal. Of the latter 10 are of type (a), 2 of type (b).

G. 1. 8 : iv : '13 (a) (Text-fig. 1, Pl. 11, fig. 19).—The operculum is growing back over the external gills, the tail and fin well-developed. The head is somewhat warty and wrinkled.

Sections show that the anterior head ectoderm is vacuolated, as are also the olfactory pits, the brain, the Gasserian ganglia, the suckers

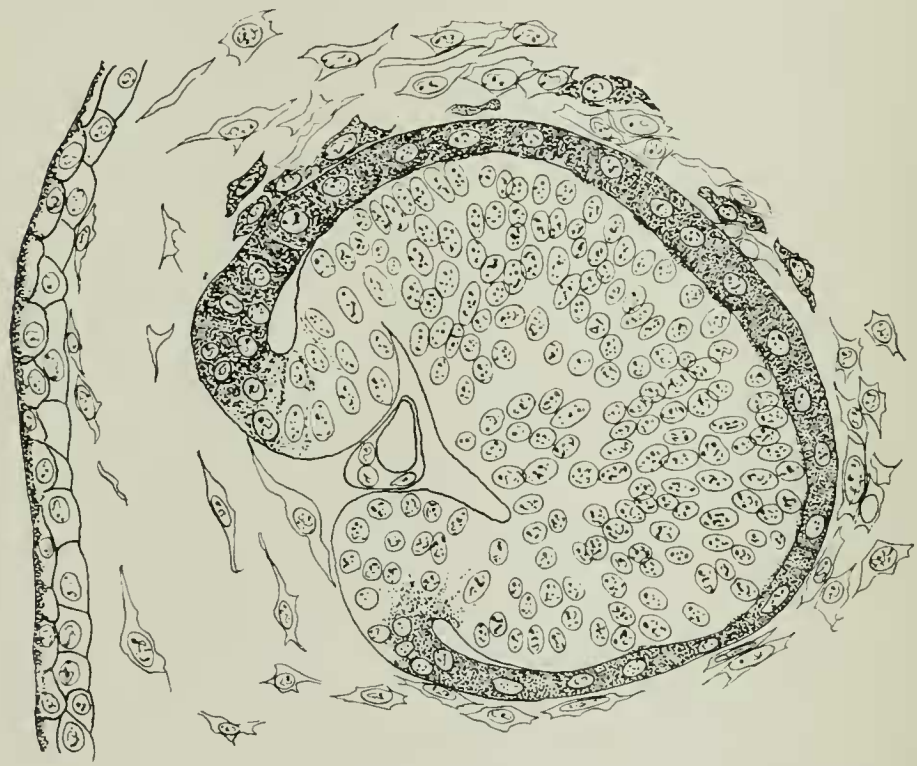
and the wandering mesoderm cells. The anterior end of the brain is single.

The optic cup is some distance from the ectoderm; its cavity is small and encloses a blood-vessel (vitreous body).

There is no lens. The corneal mesoderm is present, but the conjunctiva is not cleared. The infundibulum is present and the pituitary body. The auditory vesicles are well formed.

The notochord begins behind the infundibulum: its structure is normal.

TEXT-FIG. 1.



G. 1. 8 : iv : '13 (a). The lens-less eye. There is a blood-vessel in the small cavity of the optic cup. Corneal mesoderm and conjunctival ectoderm, choroid and sclerotic mesoderm are shown.

Differentiation of the skull and visceral arches has begun, the labial cartilages, Meckel's cartilage, the trabeculæ with the anterior trabecular plate and cornua, the quadrate, the parachordals, the hyoid and branchial arches being all present.

The thyroid is already separated, the trachea and lungs have been formed. There are external gills, and the internal gills are beginning to appear.

The heart is normal. Aortæ and cardinal veins present.

The pronephros has the usual three funnels on each side and a glomus; the ducts open to the cloaca.

The gut is normal.

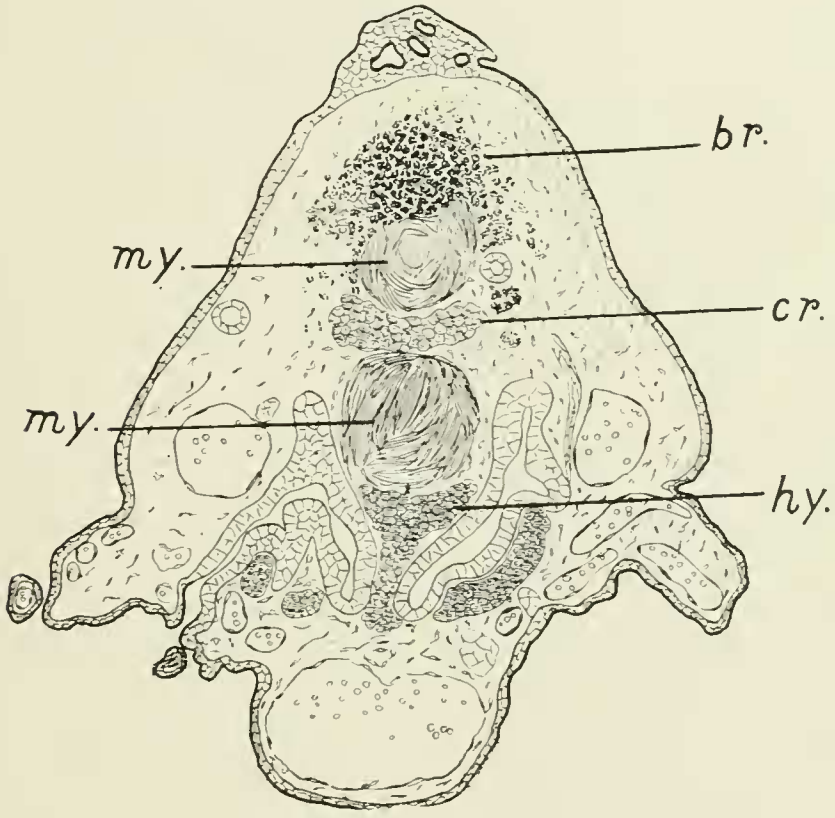
There is a blastema for the pelvis and hind limbs.

Germ-cells are present at the root of the mesentery.

The tadpole is clearly abnormal only in the vacuolation of the anterior ectoderm and its derivatives and in the absence of the lens.

G. 1. 8 : iv : '13 (b) (Text-fig. 2, *a–d*, Text-fig. 4, Pl. 11, figs. 20, 44*a*, 45, 47).—The tail is as well-developed as in the last, but the anterior end

TEXT-FIG 2*a*.



Section of the series through the tadpole, G. 1, 8 : iv : '13 (b). Through the degenerate brain represented by a mass of pigment-cells (*br.*). The rudimentary cranium (*cr.*), the hyoid (*hy.*) and parts of the branchial arches are also shown. Between the parts of the skeleton are bundles of myoblasts (*my.*). The section also shows gill-cleft, external gills, blood-vessels, a large lymphatic ventrally, and dorsally thickened and pitted ectoderm.

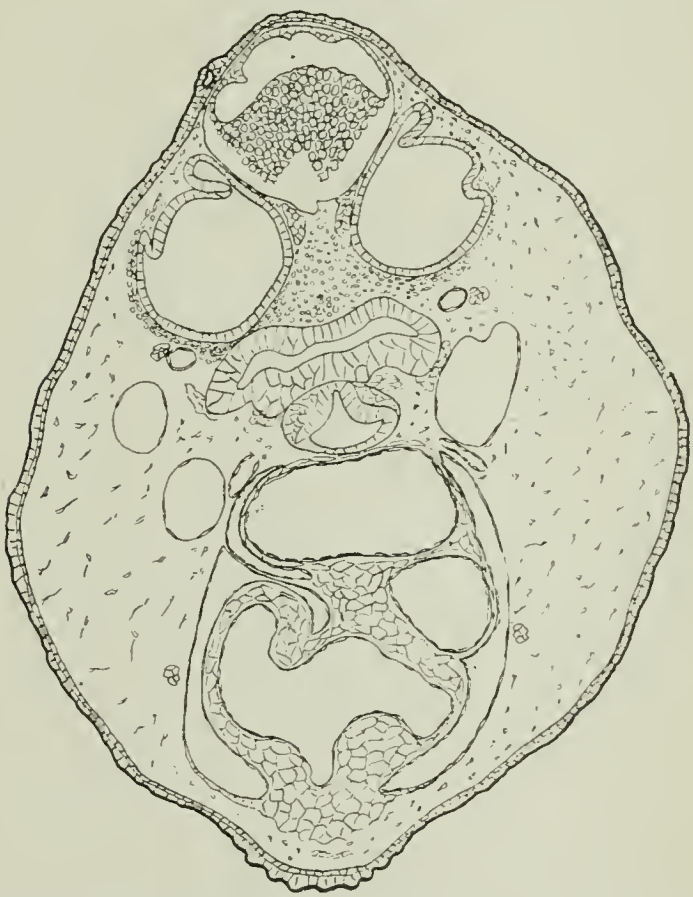
is seriously affected. The head appears to be abruptly truncated, the gills far forwards. The body is swollen. There are no suckers.

Sections show the ectoderm of the head to be folded and much vacuolated.

Olfactory pits, fore-brain, eyes, mid-brain, and part of the hind-brain are all absent, or represented only by masses of degenerating cells.

In the front part of the head is found an aggregation of pigmented and vacuolated cells, some still containing yolk-granules. These cells are the disintegrated residue of the anterior part of the nervous system, for some have the large pale nuclei characteristic of neuroblasts, others the smaller, darker nuclei of spongioblasts. Groups of cells with nerve-fibres proceeding from them are the ganglia of the fifth and seventh

TEXT-FIG. 2*b*.



Section of the series through the tadpole, G. 1, 8 : iv : '13 (b). Hind-brain, with folded roof, auditory vesicles and ganglia, œsophagus, trachea, heart, blood-vessels (aortæ and cardinals). No notochord.

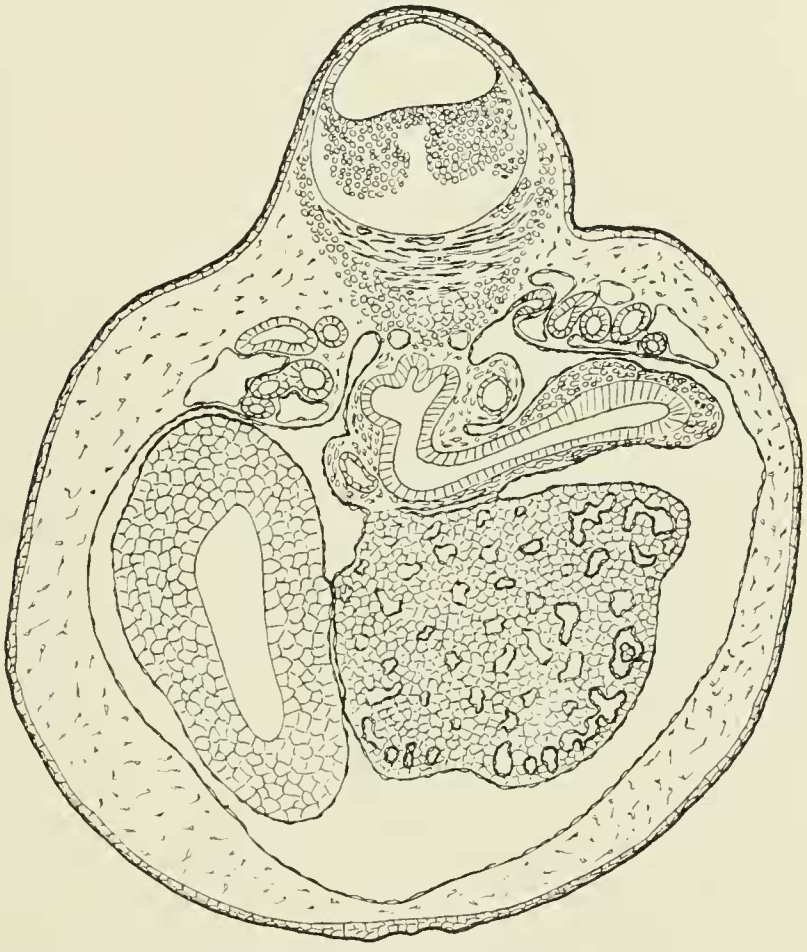
nerves. One or two small vesicles of doubtful significance are also found. In addition to the intact cells are cell-fragments, and chromatic spherules, the remains of nuclear degeneration.

In and around this accumulation are mesoderm cells, some vacuolated, others deeply pigmented (chromatophores).

The auditory vesicles are large and apparently normal; at their level begins that part of the central nervous system which has escaped des-

truction, namely the medulla, continued behind into the spinal cord. Even this, however, is not normal. In outline it is circular, its lumen crescentic. The thin roof is excessively folded, while in the thick floor there is a continuous mass of white matter across the middle line below; next to this comes a layer of neuroblasts and next the lumen a layer of

TEXT-FIG. 2 *c*.



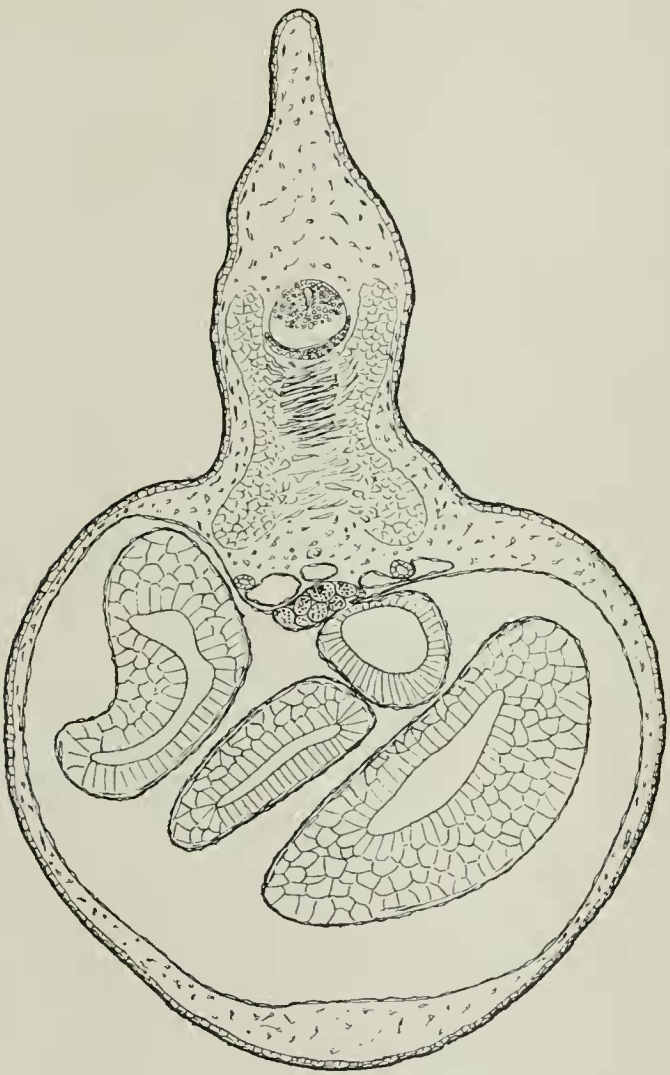
Section of the series through the tadpole, G. 1, 8 : iv : '13 (b). Pronephros (one funnel on right), stomach, liver, intestine, aortæ, cardinals. Fusion of myotomes below medulla. No notochord.

spongioblasts. The spinal cord presents the same histological characters, the lateral tracts of white matter having been apparently forced down into a median ventral position.

The ganglia of the eighth and ninth and tenth nerves are present. The spinal ganglia are abnormal in position, being united ventrally below the cord, an effect, presumably, of the same cause to which the peculiar structure of the cord is due.

The skull has also suffered, being represented only by a small bilateral plate ventral to the degenerate brain, possibly the anterior trabecular plate. Of the visceral skeleton there is a wedge-shaped piece in front of the gut, which may be interpreted as first branchial arch, with

TEXT-FIG. 2 *d*.



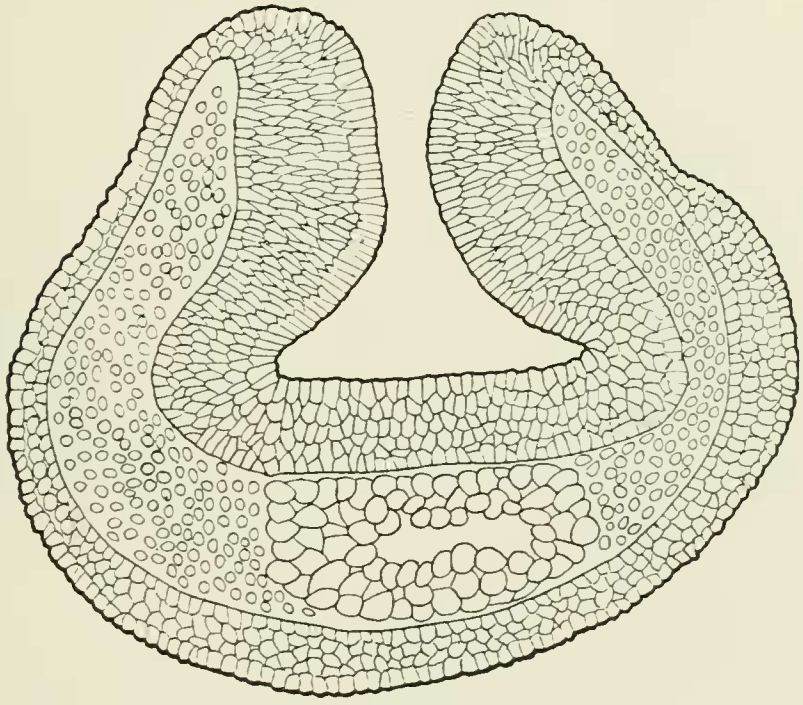
Section of the series through the tadpole, G. 1, 8 : iv : '13 (b). Pronephric ducts, primordial germ-cells, intestine. Spinal cord abnormal, with white matter in a continuous ventral band, and ganglia fused below. Myotomes fused. No notochord.

possibly mandibular and hyoid elements included in it, and behind this, underneath the throat, a basi-branchial bearing two pairs of arches; the relation of these to the gill-slits proves them to be the second and third of the series. Attached to these skeletal elements are masses of myoblasts.

There is no stomodæum, and the fore-gut is in communication with the exterior only by the gill-slits, of which the usual four are present, the last three being open. There is a rudimentary sixth cleft (pharyngeal outgrowth). External gills are borne on the first three branchial arches.

The thyroid, still connected with the throat, lies in a depression of the basi-branchial. The trachea and lungs have been formed. The heart is normal, aorta and cardinal veins present. In front of the pericardium

TEXT-FIG. 3*a*.



Optic vesicles and fore-brain. The pharynx below. I. 1, 3 : iv : '13 (c). Normal.

is a large sinus, lymphatic, partially divided by a septum. It causes the body-wall to protrude. The gut is normal.

The pronephros is well-formed, but on one side there are only two nephrostomes. The ducts open to the cloaca. There is a glomus. The notochord is very imperfectly developed. The myotomes bend round and fuse in a median mass of cells (myoblasts) below the spinal cord; in this mass notochordal tissue may be here and there distinguished, but it is feebly differentiated. Even when present it is not immediately below the spinal cord, but separated from the latter by myoblasts. Germ-cells are found at the root of the mesentery.

2. One hour after insemination, centrifuged for 10 minutes.

The grey patch round the animal pole is more conspicuous.

Segmentation, the first two furrows, may be normal, but is sometimes abnormal.

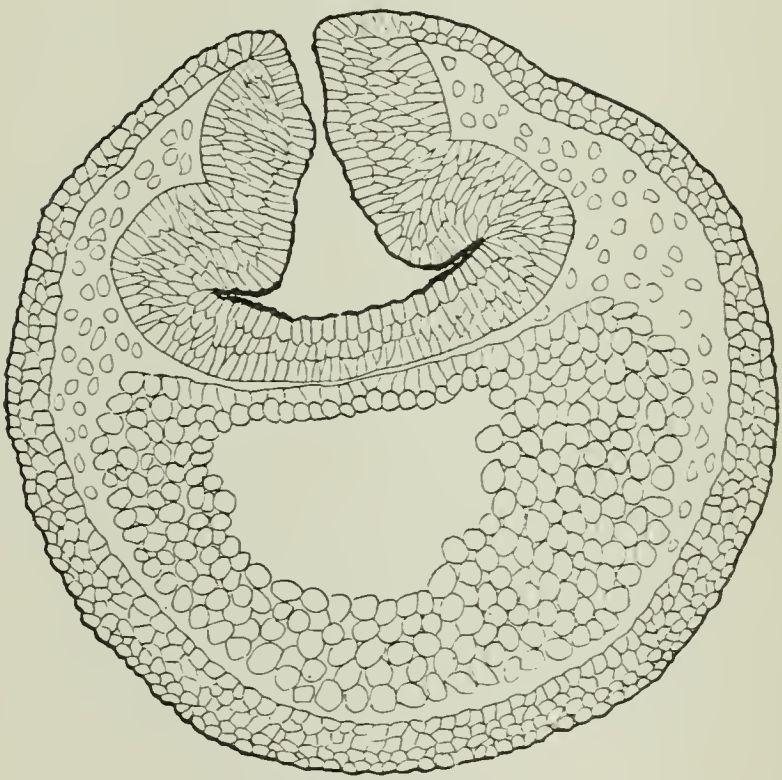
28 : iii : '13.—Segmentation has been normally completed. The grey patch is still visible.

8 : iv : '13.—Many of the tadpoles are retarded in development and have failed to hatch. Those which have hatched are inert.

All were preserved in picric acid.

Of these tadpoles 28 are normal in appearance, 15 abnormal.

TEXT-FIG. 3 *b*.



I. 1, 3 : iv : '13 (a). Optic vesicles and fore-brain. Lumen reduced. The pharynx below.

Of the latter 6 of type (a) resemble externally G. 1. 8 : iv : '13 (a), 4 of type (b) resemble G. 1. 8 : iv : '13 (b), while types (c), (d), (e), (f) and (g) are represented by one each. Suckers and mouth are absent.

G. 2. 8 : iv : '13 (a) (Pl. 12, fig. 46).—The head ectoderm is considerably vacuolated, and so are the mesodermal cells.

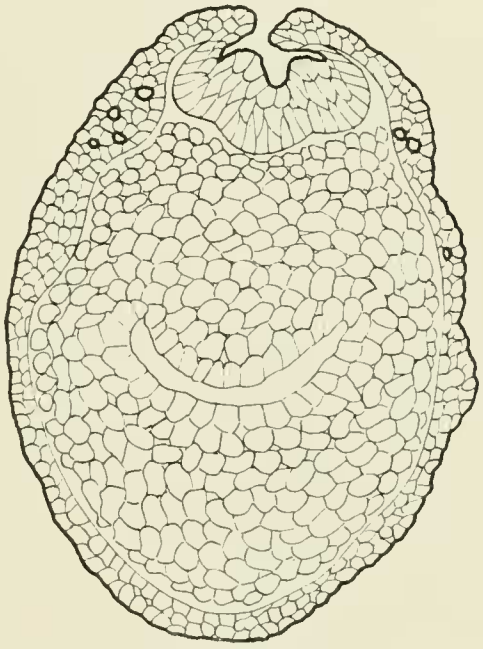
There are no olfactory pits, but two masses of vacuolated cells are found, with nerve-fibres differentiated, each hollow, and connected with the ectoderm by cell-strands.

The fore- and mid-brains and eyes are represented by paired and

median masses of pigmented vacuolated cells. Some of the nuclei have the characters of those of neuroblasts, and nerve-fibres can be seen. The remains of the fifth and seventh ganglia and nerves are also distinguishable.

The brain begins as a solid mass with longitudinal and transverse fibres at this level, and immediately behind, opposite the auditory vesicles, a lumen appears. The hind brain and spinal cord have the structure seen already in G. 1. 8 : iv : '13 (b). The spinal ganglia are

TEXT-FIG. 3 *c*.



L. 1, 6 : iv : '13 (a). Optic vesicles and fore-brain. Very much reduced. The pharynx below. The ectoderm (epidermis) is thickened and pitted.

pressed down and may meet below. The ganglia of viii and ix and x are present. The auditory vesicles are large, the cells vacuolated.

There is no trace of the trabeculæ, but the visceral skeleton is represented by a median plate below the throat bearing three pairs of branchial arches—better developed on one side than on the other—and produced in front into a ring-shaped cartilage which seems to represent the quadrate. To this skeleton are attached bundles of myoblasts, especially anteriorly. Of gill-clefts, the hyo-mandibular and first two branchials (the second open) are present on one side, the first three branchials on the other (the second and third open). There are external gills.

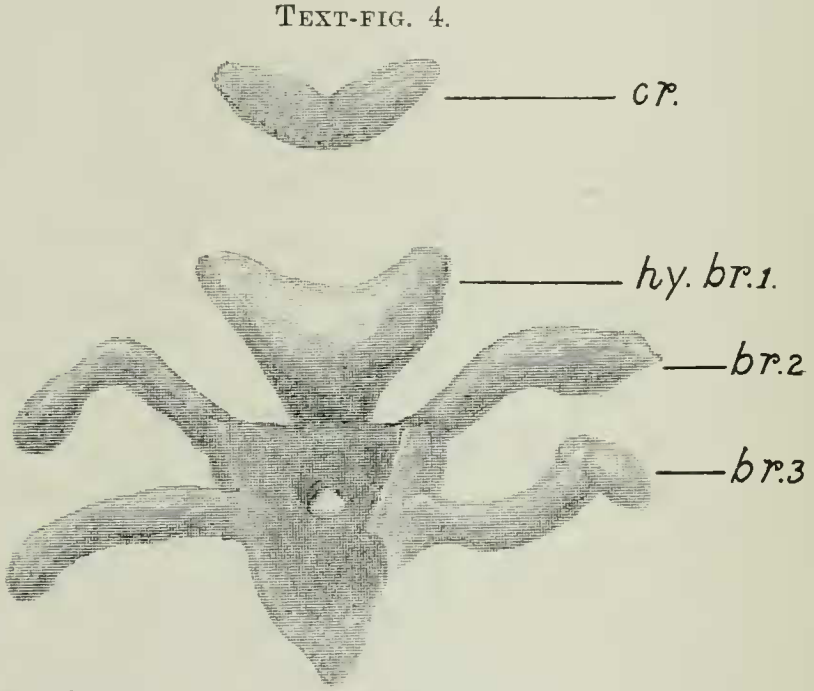
The trachea and lungs have been formed, the heart is small but

twisted. Aortæ and cardinal veins are present. There are three pronephric funnels on one side, but only two on the other. The ducts open into the cloaca. The glomus is absent.

The gut is normal, but the intestine is not yet coiled. There are germ-cells at the root of the mesentery. The myotomes bend down and meet below the spinal cord. In this mass is the notochord, imperfectly differentiated in front, more posteriorly vacuolated. More posteriorly still the notochord is free and the myotomes separate.

The whole body is very œdematous; the connective tissue and the

TEXT-FIG. 4.


cr.
hy. br.1.
br.2
br.3

Reconstruction of the cranium (*cr.*) and visceral skeleton (*hy. br.* 1, *br.* 2, *br.* 3) of G. 1, 8 : iv : '13 (b).

posterior cardinal vein round the pronephros suffer especially from this accumulation of fluid.

G. 2. 8 : iv : '13 (b) (Text-figs. 8, 11).—Anteriorly the ectoderm is highly vacuolated; so also are the mesodermal cells.

Ventrally the ectoderm is also folded for some little way back.

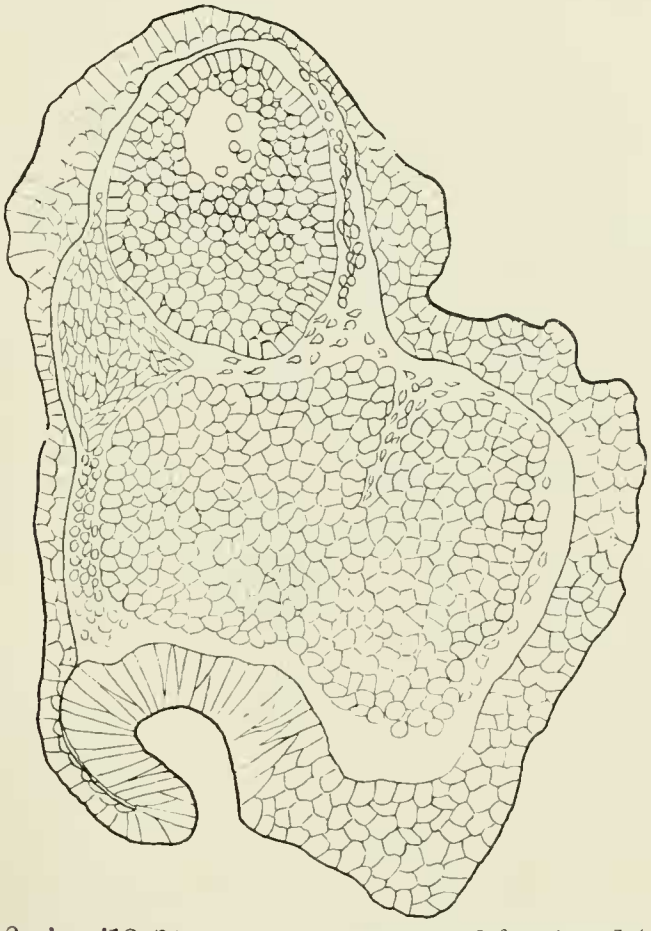
There are no suckers and no mouth. The gills are far forwards. Olfactory pits, eyes, fore- and mid-brains represented only by a heap of pigmented vacuolated cells, with traces of fibres.

The auditory vesicles with ductus endolymphatici. At this level the brain begins. The hind-brain and the spinal cord have the same characters as in (a), but the posterior part of the spinal cord is normal. The auditory, vagus, and spinal ganglia are present, the spinal ganglia being united ventrally.

The visceral skeleton—the only skeleton developed—consists of a median plate bearing three arches, the first, second and third, and an anterior prolongation, situated in the front wall of the pharynx, which represents hyoid and perhaps quadrate elements.

The hyomandibular cleft is absent. The first, second, third, and fourth branchial clefts are present on both sides, the fourth being just open on one side, while on the other the second and fourth are open.

Text-fig. 5.



H. 3. 2 : iv : '13 (b). Not very abnormal front end (section obliqne). Brain, neural crest, pharynx, one sucker cut.

There are external gills.

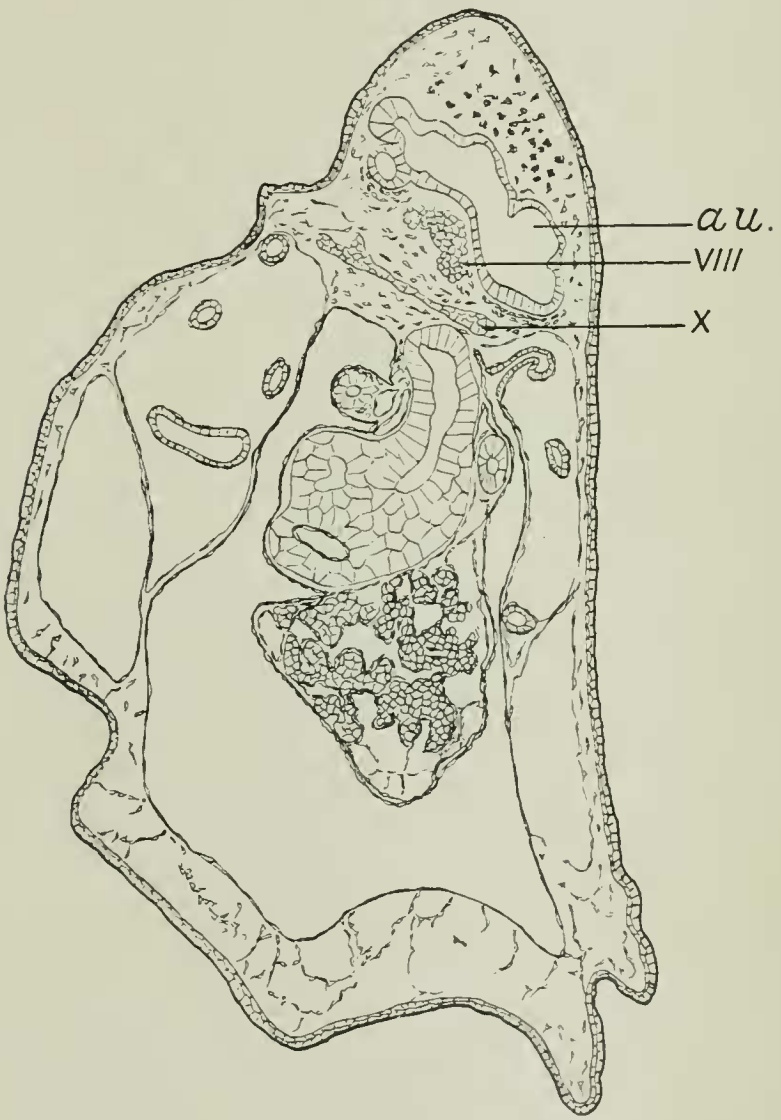
The trachea and lungs are formed, but the trachea is solid.

The heart is also solid, small, and hardly twisted. Aortæ and cardinal veins are formed. The pronephros has three funnels on each side, and a glomus. The ducts open to the cloaca.

The intestine is not differentiated into regions, and in the yolk-cells is a central mass where there are no cell-divisions and the granules are fused into a coagulable liquid.

Germ-cells are found at the root of the mesentery. The myotomes are fused below the spinal cord. The notochord is only distinguishable behind the pronephros. Here it lies immediately ventral to the spinal cord.

TEXT-FIG. 6.



G. 2. 8 : iv : '13 (g). Degenerate brain (pigmented cells). The auditory vesicles united (*au.*); below them the auditory and vagus ganglia (*viii*, *x*) also united. Œsophagus, lungs, liver, pronephros (one funnel cut on right). Œdematous connective tissue.

In the cœlom and in the posterior cardinal vein round the pronephros is an accumulation of fluid.

G. 2. 8 : iv : '13 (c) (Text-fig. 10, Pl. 11, fig. 21).—Body much

swollen; tail well developed. Neither mouth nor suckers. The head ectoderm is vacuolated and the mesoderm. The mesoderm s œdematous. Olfactory sacs, eyes and front part of brain represented by an aggregation of pigmented and vacuolated cells, with traces of the ganglia of v and vii, but no nerve-fibres. Hind brain, spinal cord and spinal ganglia as in (a); the spinal cord abnormal to the end.

The auditory vesicles and the auditory and vagus ganglia are present.

A small nodule of perichondrium in the front wall of the pharynx is

TEXT-FIG. 7.

I. 1. 4 : iv : '13 (d). Auditory invaginations. The solid wedge of cells in the middle of the larger endoderm cells is the degenerating fore- and mid-brains. Ectoderm thick and pitted (cut somewhat tangentially on the ventral side).

the only representative of the visceral skeleton. There is no trace of the cranium.

The pharynx has an irregular cavity, but there are no gill-clefts.

The trachea and lungs are present, but the latter are short.

There is no heart.

The pronephros has only two funnels on each side, and there is no glomus. The ducts are open to the cloaca.

The stomach and duodenum are very small.

Germ-cells are present in the mesentery.

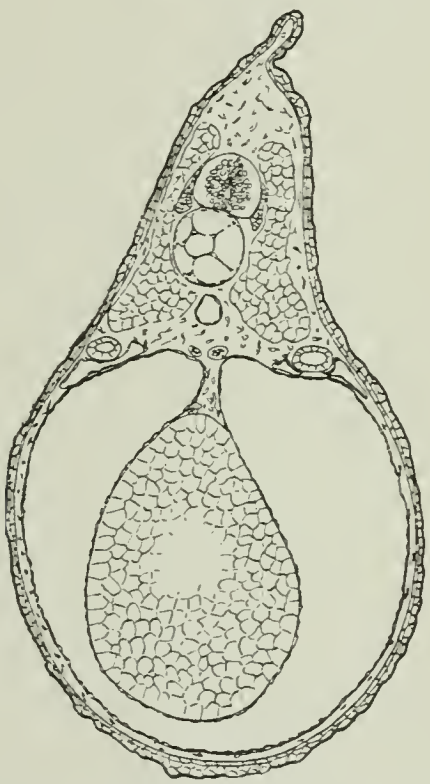
The myotomes, which are fused below the spinal cord, are poorly developed in front.

There is no notochord except in the tail.

There is great œdema of the connective-tissue spaces, of the posterior cardinal veins round the pronephros, of the cœlom, and of spaces (blood-vessels or lymphatics) around the stomach and duodenum.

The ectoderm is distended and flattened.

TEXT-FIG. 8.



G. 2. 8 iv : '13 (b). Hind-end, normal, except for central mass of undivided yolk in intestine and absence of lumen.

G. 2. 8 : iv : '13 (d) (Text-fig. 14 *a*, *b*, Pl. 11, fig. 22).—The body is very stunted; there is a short tail. The blastopore is widely open, and the yolk-plug protrudes.

At the front end the ectoderm is folded, pitted and highly vacuolated.

Internally the front end is occupied by a large cavity with a lining of flattened mesoderm cells. The cavity is probably persistent blastocœl. The hinder wall of this space is occupied by a mass of undifferentiated mesodermal and yolk-cells. Further back are traces of cœlom and blood-vessels.

There is no sign of nervous system, notochord, or myotomes.

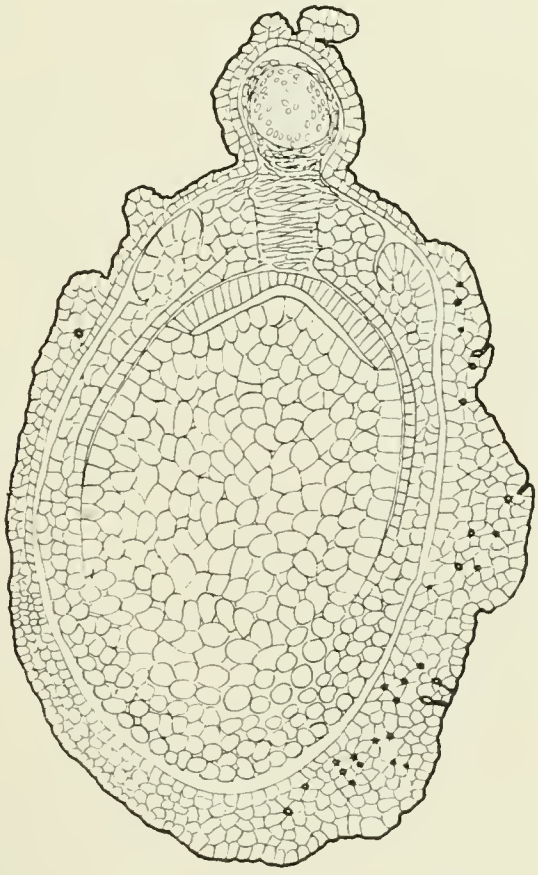
G. 2. 8 : iv : '13 (e) (Text-figs. 12, 13, Pl. 11, fig. 23, Pl. 12, fig. 44 *b*). —Body short; short tail; persistent large yolk-plug. No mouth; no suckers.

Ectoderm of head folded and wrinkled; highly vacuolated.

No olfactory pits nor eyes; no fore- nor mid-brain. These structures are represented by a mass of pigmented cells.

Ganglia of v and vii not to be found. The auditory and vagus ganglion pairs each united across the middle line.

TEXT-FIG. 9.



I. 1. 4 : iv : '13 (d). Thick and pitted ectoderm. Spinal cord solid. Notochord not properly differentiated. Pronephric ridge.

Auditory vesicles well formed, each constricted into two cavities.

The brain begins at the level of the auditory vesicles; it is solid, with fibres ventrally.

The spinal cord has similar characters, but a lumen appears in it here and there. The spinal cord does not reach the hind end of the body. The spinal ganglia are united below.

Fore-gut, but no gill-slits. No lungs.

Gut not much differentiated. In the yolk-cells a central mass without cell-boundaries; here the yolk-granules are fused.

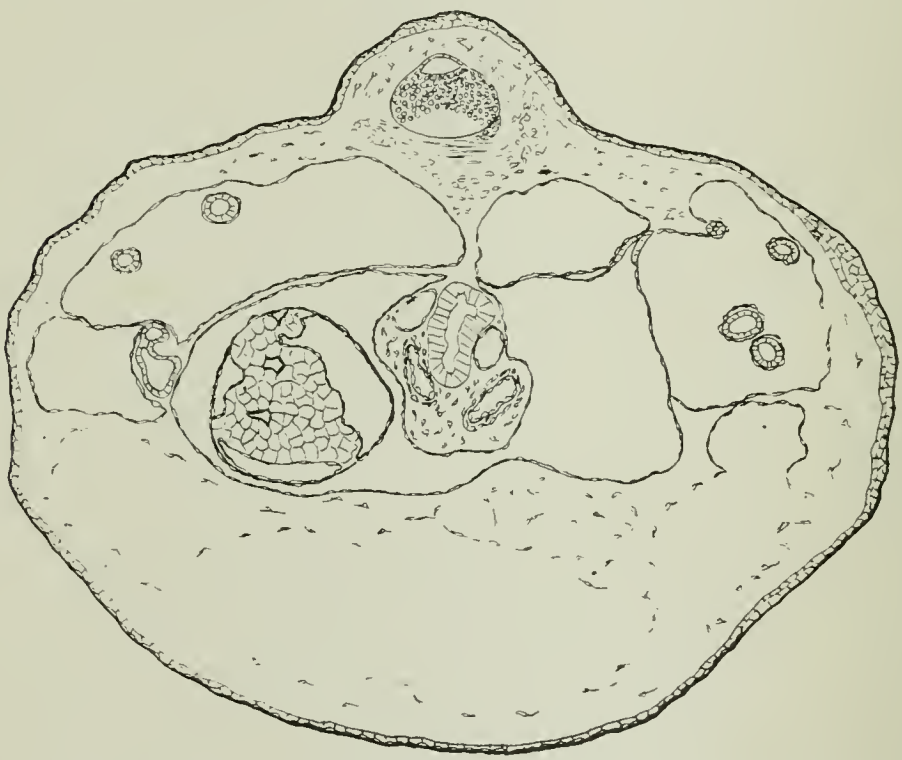
Blood-corpuscles are being formed from the yolk-cells ventro-laterally.

A pericardium is present, but the heart is represented only by a solid cell-mass projecting into this cavity from above. Vitelline veins and cardinal veins are present.

The pronephros has three funnels on each side, and a small glomus. The ducts end blindly.

The pronephric tubules are enormously swollen.

TEXT-FIG. 10.



G. 2. 8 : iv : '13 (c). Pronephros, one funnel cut on right. Abnormal medulla. Fusion of myotomes; no notochord. Œsophagus. Lungs. Liver. Enlargement of posterior cardinal vein and of lymphatics round liver. Œdematous connective tissue.

The myotomes are united across the middle line by a mass of fusiform myoblasts, some of which are vacuolated. No notochord, except for an occasional vacuolation.

The peritoneal cavity is well developed.

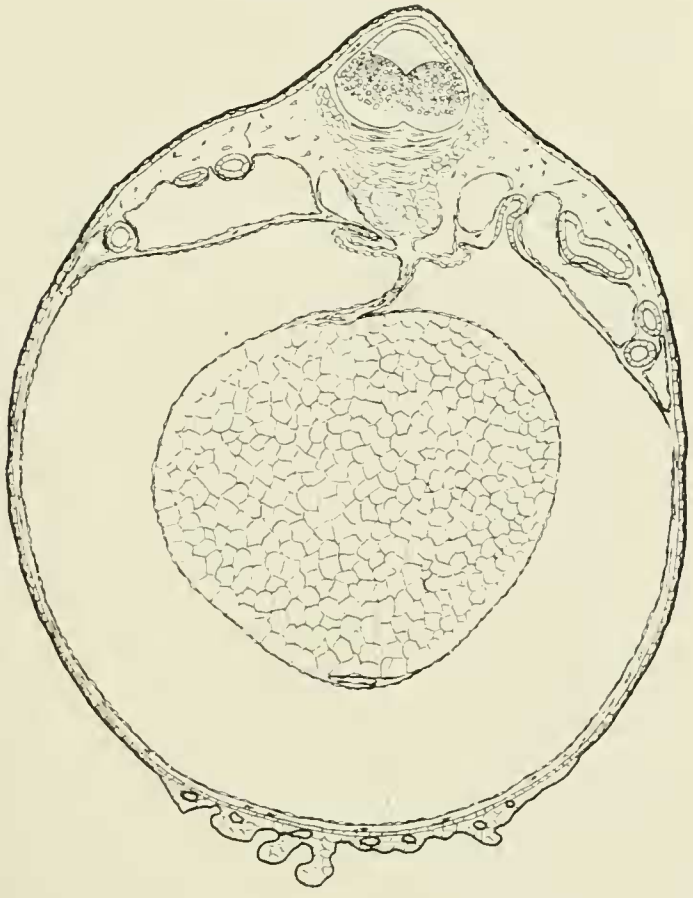
G. 2. 8 : iv : '13 (f).—Body very short and undifferentiated. At one end, presumably anterior, the ectoderm is highly vacuolated and wrinkled. At this end is what appears to be a yolk-plug, but is in reality a yolk-burst.

There is a small archenteron posteriorly opening by the blastopore, a rudimentary pericardium, and the mesoderm is to some extent differentiated—as myoblasts, connective tissue and blood-corpuscles.

No trace of nervous system, nor of sense-organs, nor of notochord, nor of pronephros.

G. 2. 8 : iv : '13 (g) (Text-fig. 6).—Body short, neither mouth nor

TEXT-FIG. 11.



G. 2. 8 : iv : '13 (b). Pronephros, one funnel cut on right, glomus. Cœlom much enlarged. Myotomes fused below medulla. Ventral ectoderm folded.

suckers. Tail with dorsal and ventral fins. There is a side branch to the tail but this does not receive any spinal cord.

The anterior ectoderm is much vacuolated. Neither olfactory pits nor eyes. Fore- and mid-brains represented by a mass of vacuolated pigmented cells. Ganglia of v and vii present, with the nerves developing.

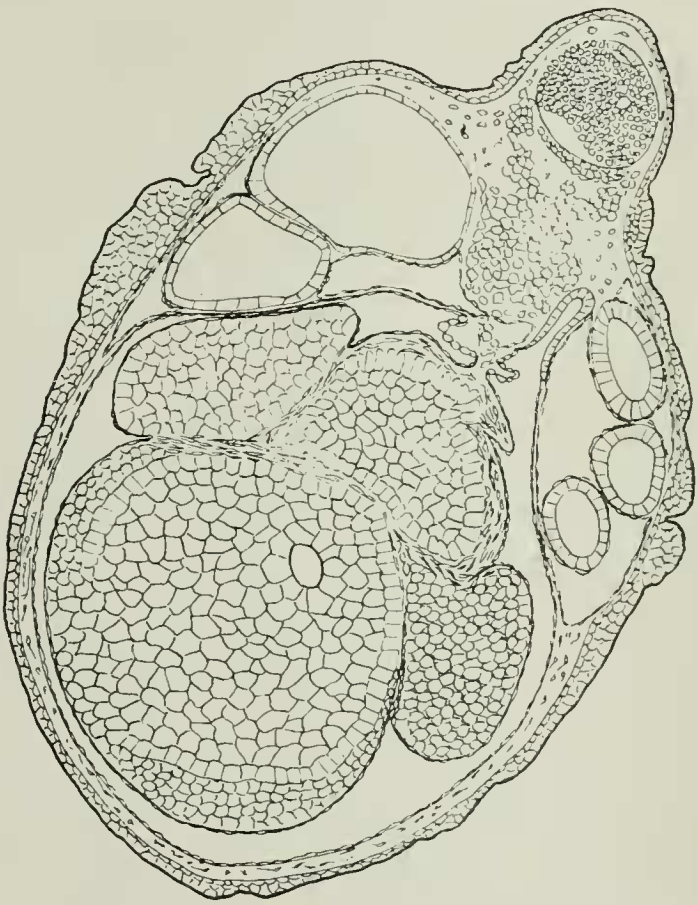
Auditory and vagus ganglion pairs each united below the brain.

The hind-brain, with fibres and neuroblasts stretching across the

thick floor, begins behind the auditory vesicle; the thin roof is much folded. The spinal cord, which has similar characters and a small lumen, is continued into the tail. The ganglia are united below the cord.

The auditory vesicles are large and united, with their cavities also in communication, above the hind-brain.

Text-fig. 12.



G. 2. 8 : iv : '13 (e). Spinal cord nearly solid. Pronephric tubules much swollen, especially on left.

There are external gills, and gill-clefts, two on one side, probably the first and second branchial, the second being open, and three on the other sides, probably the first, second and third branchial, the last two being open.

The visceral skeleton is represented by two pieces: one, anterior to the pharynx and even dorsal to it, probably represents the hyoid and first branchial arches. Muscles (myoblasts) are attached to it. The other is below the pharynx and bears a pair of arches, probably the second branchial.

Trachea and lungs.

Heart and pericardium, the heart straight.

Pronephros with three funnels on each side, the ducts open. The glomus much enlarged.

Stomach and intestine differentiated, liver present, proctodæum open. A central mass of fused granules in the yolk of the intestine.

The myotomes are united below the medullary tube by elongated myoblasts, but there is no trace of a notochord.

Germ-cells at the root of the mesentery.

The connective tissue is œdematous and the posterior cardinal veins much distended.

3. One and a half hours after insemination, centrifuged for 17½ minutes.

A whitish-yellow ring appears round the grey patch.

Segmentation in early stages is normal, except for the inequality of the first or second division, or both.

28 : iii : '13.—A grey and yellow patch is still visible. The animal hemisphere is segmented in some, but not in all. The vegetative hemisphere is segmented in none.

8 : iv : '13.—All the eggs are dead, undeveloped.

4. One and a half hours after insemination, centrifuged for 28 minutes.

As the last; folds appear in the grey patch.

Segmentation is more abnormal than in the last.

28 : iii : '13.—Like the last.

8 : iv : '13.—All the ova dead, undeveloped.

H.

Centrifuged, 28 : iii : '13, on the water-driven machine, about one hour after insemination.

1. For 10 minutes at speed III.

A grey and yellow patch, with folds, appears round the animal pole.

29 : iii : '13.—The yellowish patch is still present. The animal hemisphere is segmented, but the vegetative is not; it is blotched with pigment.

30 : iii : '13.—No blastopore has appeared.

2. For 10 minutes at speed II.

There is a grey patch surrounded by a yellowish ring.

29 : iii : '13.—The animal hemisphere, which still shows the grey patch, is segmented, but the vegetative is not; it is blotched with pigment.

30 : iii : '13.—There is no blastopore.

3. For 10 minutes at speed I.

A grey patch appears round the animal pole, but there is no yellowish margin.

29 : iii : '13.—The grey patch is still present.

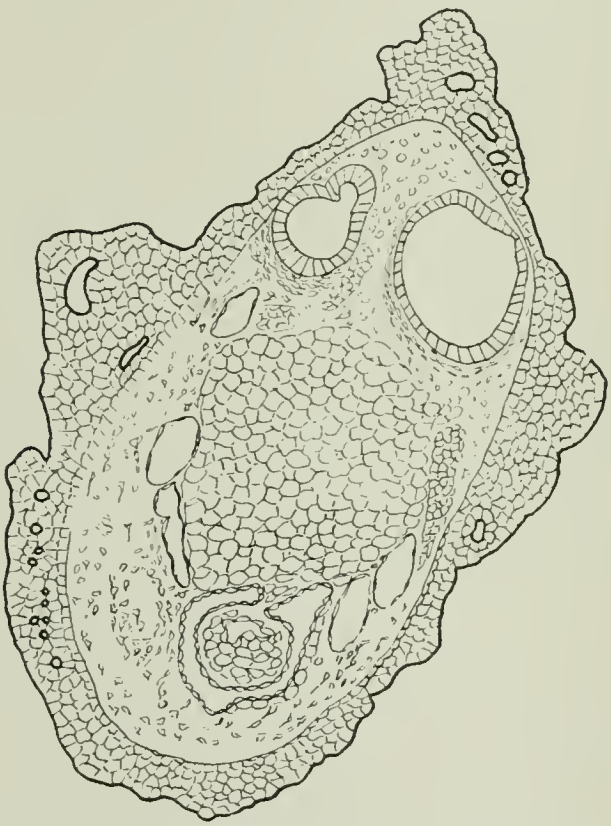
The animal hemisphere is well segmented. The vegetative hemisphere is also certainly segmented in some of the ova. It is blotchy.

30 : iii : '13.—A semicircular blastopore is present.

2 : iv : '13.—Four of the embryos are dead in an early stage. Of the others, some are normal, some abnormal, and of two types, (a) and (b). All these embryos were preserved in picric acid.

H. 3. 2 : iv : '13.—The normal embryos.

Text-fig. 13.



G. 2. 8 : iv : '13 (e). Heart small, fore-gut solid. Auditory vesicles well-formed. Ectoderm very thick and pitted.

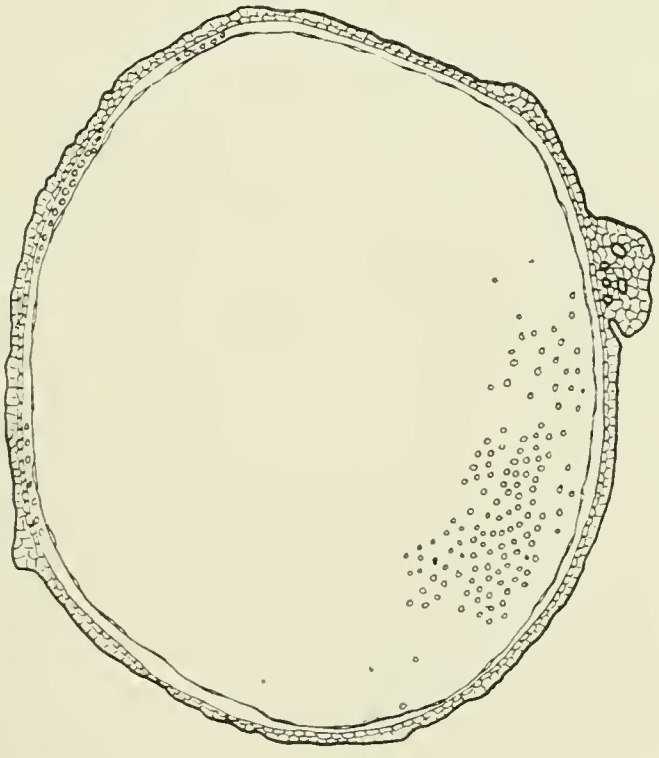
There is a short tail stump. Stomodæum and proctodæum. Suckers. Sections show olfactory pits, optic vesicles, lens thickenings, auditory vesicles in an early stage of invagination, endothelial cells of heart, paired pericardium, pronephric ridge, sclerotome, notochord and sub-notochordal rod. The only abnormality is the mass of fused granules in the centre of the yolk-cells of the gut.

H. 3. 2 : iv : '13 (a) (Pl. 11, fig. 24).—The front end is normal, with olfactory pits, optic vesicle, lens thickening, auditory vesicle, stomodæum, pituitary body, suckers, paired pericardium, endocardial

cells, pronephric ridge, sclerotome, notochord and subnotochordal rod. In the centre of the yolk is a mass of fused granules. Posteriorly there is an exposed yolk-plug bounded above by the tail—containing spinal cord, notochord and mesoderm—and in front and on the left by a small knob which may be a second tail, inasmuch as it contains two rounded cell masses and some mesoderm.

H. 3. 2 : iv : '13 (b) (Text-fig. 5, Pl. 11, fig. 25).—The anterior end is abnormal. The ectoderm here is highly vacuolated, the olfactory pits

TEXT-FIG. 14 *a*.



G. 2. 8 : iv '13 (d). Section of front end through the enlarged head-vesicle lined by a thin layer of mesoderm and containing some scattered mesoderm cells. This is the persistent segmentation cavity.

very shallow, the fore- and mid-brain represented by a solid mass of vacuolated cells, and there is no sign of the infundibulum nor of the optic vesicles. The neural crest goes forwards into this region. The lumen of the medullary tube appears first in the hind-brain just in front of the auditory vesicles, and is continued into the spinal cord, which is normal.

The auditory vesicles are just invaginated, ductus endolymphatici being present.

There is no stomodæum; the suckers are very far forwards.

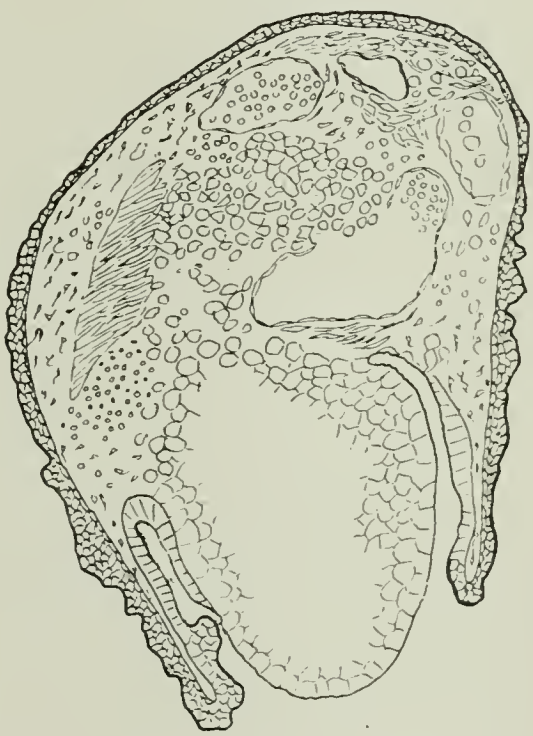
Fore-gut with incipient gill outgrowths, but no clefts.

Heart-tube between the two sides of the paired pericardium. Slight peritoneal cavity, pronephric ridge, sclerotome, notochord and subnotochordal rod.

What appears to be an enlarged liver outgrowth extends forwards below the heart.

A mass of fused yolk-granules in gut. Hind-end normal with tail gut and neurenteric passage (a streak of pigmented cells).

TEXT-FIG. 14 *b*.



Posterior end of the same as Text-fig. 14 *a*, through the yolk-plug, with deep blastoporic involution. Some myoblasts and blood-vessels have been differentiated.

I.

Centrifuged 31 : iii : '13 on the water-driven machine at speed IV.

1. Fifty minutes after insemination, centrifuged for 10 minutes.

A grey patch appears round the animal pole; it is surrounded by a yellowish-white ring, and in its centre is a yellowish spot. Segmentation is normal.

2 : iv : '13.—The grey patch is still visible. There is a crescentic blastopore.

3 : iv : 13.—The grey patch is still present.

Medullary folds formed, blastopore widely open and yolk-plug protruding. Three embryos preserved, (a), (b) and (c).

I. 1. 3 : iv : '13 (a) (Text-fig. 3*b*, Pl. 11, fig. 26).—The anterior ectoderm thickened and vacuolated. The brain-cells are also vacuolated. The optic vesicles are abnormally thick-walled, and have an abnormally narrow lumen.

Nerve crest, sense-plate and gill-plate present. The notochord is distinct.

In the yolk is a central mass of fused granules. The yolk-plug is lateral (the blastopore has closed on the right, and the medullary tube has grown back).

I. 1. 3 : iv : '13 (b) (Pl. 11, fig. 27).—Anterior ectoderm thickened and vacuolated. The medullary groove shallow, deeper in front, but the optic vesicles have not yet been evaginated. There is a neural crest. The notochord is hardly distinct from the mesoderm.

There is a central mass of fused yolk-granules in the gut.

I. 1. 3 : iv : '13 (c) (Text-fig. 3*a*).—The anterior ectoderm and brain vacuolated as in (a). The optic vesicles have a lumen of nearly normal size. There is a neural crest, and the notochord is beginning to be vacuolated. The vertebral plate is beginning to be separated from the lateral plate.

There is a central mass of fused yolk-granules in the gut.

4 : iv : '13.—Some are normal or nearly so, but many have large, persistent yolk-plugs.

All these embryos were preserved.

Three are apparently normal, (a), ten abnormal. Of the latter there are seven of type (b), and one each of types (c), (d) and (e).

I. 1. 4 : iv : '13 (a).—Normal except for the vacuolation of the head ectoderm and the fusion of yolk-granules in the centre of the yolk-cell mass.

Olfactory pits, optic vesicles, slight lens thickening, auditory invaginations, stomodæum, pituitary body, notochord, pharynx, gill-outgrowths, pericardium and heart-tube, suckers, liver, pronephric ridge, slight peritoneal cavity, subnotochordal rod, proctodæum and neurenteric streak of pigmented cells.

I. 1. 4 : iv : '13 (b) (Pl. 11, fig. 28).—Like (a), except for the yolk-plug.

I. 1. 4 : iv : '13 (c) (Pl. 11, fig. 29).—Head ectoderm vacuolated. The front part of the brain degenerate, but the optic vesicles can be distinguished as small, almost solid outgrowths.

Shallow olfactory pit, auditory invaginations. Suckers absent.

There are indications of a pericardium, but there is no heart.

The notochord is distinct. There is a pronephric ridge. The hind-

end is fairly normal except for the large yolk-plug. The spinal cord ends in the middle line, it does not pass into the caudal swellings.

There is fusion of yolk-granules in the centre of the yolk-cells.

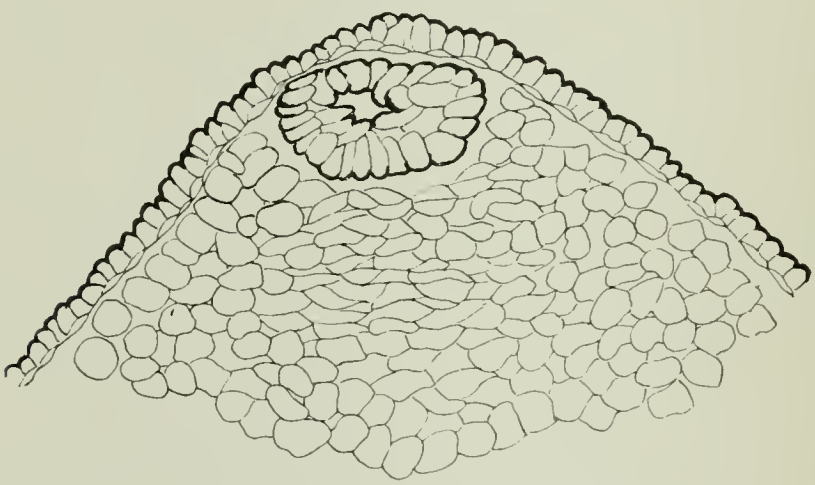
I. 1. 4 : iv : '13 (d) (Text-figs. 7, 9).—The head ectoderm is highly vacuolated, thickened and pitted.

The fore- and mid-brains are represented only by a mass of pigmented cells which are mingled with an aggregation of yolk-containing cells, the front wall of the fore-gut.

In this region are found the two auditory ingrowths.

The medullary tube begins behind the latter as a solid cord, but further back a lumen appears, nearer the dorsal than the ventral side.

Text-fig. 15.



L. 1. 6 : iv : '13 (c). Spinal cord very small. Notochord not differentiated from the mesoderm. The mesoderm of the vertebral plates united across the middle line by cells which are beginning to elongate.

The spinal cord is similar, but anteriorly the lumen is only present here and there; at the hind end it is better developed.

Suckers, heart, and pericardium are absent.

The neural crest is lateral, not ventral.

The pronephric ridge is well developed. There is no notochord; the myotomes are united across the middle line by horizontally elongated myoblasts. This median cell-mass is, like the myotomes, segmented.

The gut has a lumen. There is a mass of fused yolk-granules. The yolk-plug protrudes.

I. 1. 4 : iv : '13 (e) (Text-fig. 16, Pl. 11, fig. 30).—The anterior ectoderm is very thick, vacuolated and pitted.

No fore-brain nor mid-brain, nor even auditory vesicles. There are no suckers. The medullary tube is narrow, almost solid, and passes into one lip of the widely open blastopore, round one side and there ends. There is no notochord. Dorsal and ventral mesoderm are present, but the former is not differentiated into vertebral and lateral plates.

Neither heart nor pericardium are found, nor a pronephric ridge.

At the ventral lip of the blastopore is a short ectodermal involution, presumably the proctodæum.

The gut has a narrow crescentic lumen.

2. Seventy-five minutes after insemination, centrifuged for 30 minutes.

There appears a yellow-grey patch round the animal pole; it is much folded. Round it is a double whitish ring, and round this again a grey ring.

Segmentation is abnormal. The first and the second divisions are not meridional, and the grey patch may be cut off as a separate "cell."

Some fail to segment.

2 : iv : '13.—Only one or two have segmented and developed further; the blastopore, if present, is a wide semicircle.

3 : iv : '13.—No further development. Cellular disintegration setting in.

The zones are still distinguishable.

I. Controls, 2 : iv : '13.—Semicircular blastopore.

J.

Centrifuged, 1 : iv : '13, on the water-driven machine, at speed I, 50 minutes after insemination.

1. For 10 minutes (Pl. 7, fig. 7.)

2. For 20 minutes.

The results are similar in the two cases. A faint grey patch appears round the animal pole with a margin of a rather lighter colour.

Segmentation is perfectly normal.

8 : iv : '13.—The tadpoles are ready to hatch, and as normal as the controls.

K.

Centrifuged 2 : iv : '13 on the water-driven machine at speed II, 70 minutes after insemination.

1. For 10 minutes (Pl. 9, fig. 13).

A grey patch appears round the animal pole with central, or sometimes excentric, yellowish-white spots, and surrounded by a lighter marginal ring.

Segmentation is normal.

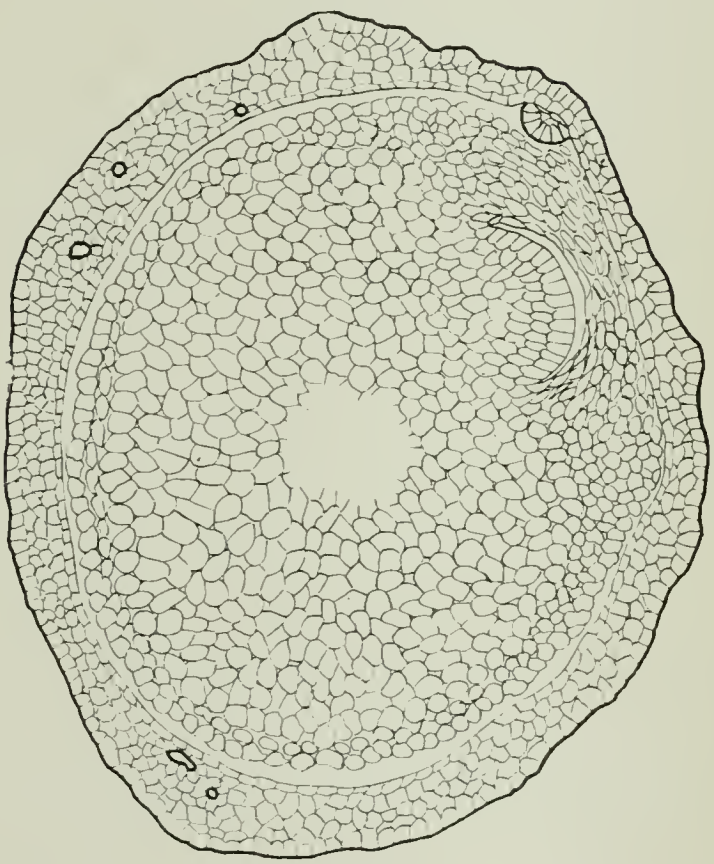
2 : iv : '13.—The grey patch is still visible. The ova appear to be as well segmented as the controls.

3 : iv : '13.—The grey patch is faintly visible. The blastopore is three quarters of a circle.

4 : iv : '13.—The medullary folds are closing. Fairly normal.

6 : iv : '13.—Fairly normal.

TEXT-FIG. 16.



I. 1. 4 : iv '13 (e). Spinal cord reduced to a minimum. Gut lumen very narrow. Dorsal and ventral mesoderm differentiated, but no notochord. Ectoderm thickened and pitted. Central undivided yolk-mass.

8 : iv : '13.—Fairly normal, with stomodæum, suckers, nostril, gills, tail and fin.

These tadpoles were not preserved.

2. For 20 minutes.

The grey patch becomes folded. The marginal ring is more marked, and may be confluent with the spots inside the grey patch.

Segmentation is not normal, the first and second furrows not being meridional, and the rate of division is retarded.

2 : iv : '13.—The animal hemisphere alone is segmented. It is streaked with white or grey. The vegetative hemisphere is blotched with pigment.

3 : iv : '13.—The blastopore has not yet appeared.

4 : iv : '13.—The embryos resemble those of I. 1. 3 : iv : '13. The medullary folds are developed, the yolk-plug is exposed. The grey patch is still to be seen at the anterior end.

6 : iv : '13.—Dead in an early stage.

These embryos were not preserved.

L.

Centrifuged 1 : iv : '13 on the water-driven machine at speed III.

1. Forty minutes after insemination, centrifuged for 5 minutes.

The grey patch round the animal pole has a whitish spot in the centre and is surrounded by a faint marginal ring.

Segmentation is normal in form but slightly retarded on the controls.

2 : iv : '13.—The grey patch and whitish spot are still visible. Segmentation has proceeded not quite as far as in the controls.

3 : iv : '13.—The grey patch is still present. In some eggs the dorsal lip of the blastopore has appeared.

4 : iv : '13.—The blastopore is circular, the yolk-plug large and protruding.

6 : iv : '13.—There is a tail stump. The blastopore is open in many.

Twelve of these embryos were preserved.

Of these, 2 are normal, with nostrils, suckers, stomodæum and incipient gill-clefts, 9 are monstrous, and 1 undeveloped. Of the 9 monstrous embryos, 1 is of type (a), 1 of type (b), 1 of type (c), 2 of type (d), 1 of type (e), 1 of type (f), and 2 of type (g).

L. 1. 6 : iv : '13 (a) (Pl. 11, fig. 31, Text-fig. 3 *c*).—Anteriorly the ectoderm is very thick and vacuolated. A solid in-growth of this represents the fore-brain, produced into minute optic vesicles.

There are traces of olfactory pits.

The in-growth of ectoderm becomes grooved behind this point, and the groove deepens : this is the mid-brain. Then the groove closes, in the region of the hind-brain.

The auditory vesicles are in an early stage of invagination.

There are no suckers.

The heart and pericardium are not formed yet.

The notochord is normal, slightly vacuolated.

The mesoderm is also normal, with somites, lateral plate and pronephric ridge.

There is a small lumen to the gut, open at the blastopore. There is no proctodæum.

The medullary tube and notochord pass to one side of the protruding yolk-plug. Neural crests small. In the yolk-cells there is a central mass of fused granules.

L. 1. 6 : iv : '13 (b).—The vacuolated anterior ctoderm is very thick and crinkled.

Neither nostrils nor suckers are present.

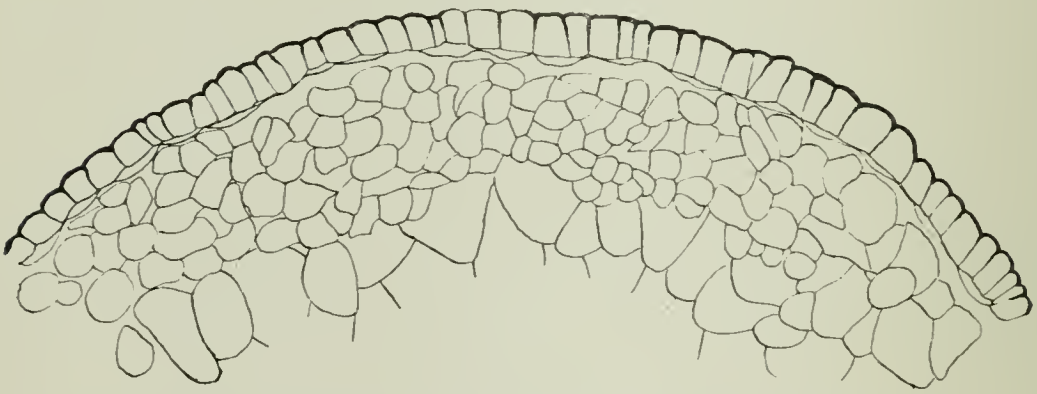
There is hardly any medullary tube in front of the auditory invaginations, and that is solid.

The cells of the hind-brain are vacuolated, the spinal cord is normal. The neural crests are small.

There are no gill-clefts.

There is a pericardium but no heart.

TEXT-FIG. 17.



L. 1. 6 : iv : '13 (g) (1). No nervous system. No differentiation of the notochord from the dorsal mesoderm.

Somites, lateral plate and pronephric ridge are present.

The notochord goes as far forwards as the auditory vesicle.

The proctodæum is open.

There is a mass of fused yolk-granules in the yolk-cells; the large yolk-plug is one-sided.

L. 1. 6 : iv : '13 (c) (Pl. 11, fig. 32, Text-fig. 15).—Anterior ectoderm thick and vacuolated.

No olfactory pits, no auditory vesicles, no suckers.

The medullary tube very asymmetrical in front; this is presumably the hind-brain, but in the absence of the auditory vesicles it is impossible to say with certainty.

The lumen of the medullary tube is very small and absent in places. Behind is an open medullary groove.

The notochord is not distinguishable in front, barely so behind.

There is a pericardium, but no heart.

The pronephric ridge is indicated.

The fore-gut is much folded and crumpled.

The gut lumen disappears behind.

There is the usual mass of fused granules in the middle of the yolk-cells, and a large yolk-plug.

L. 1. 6 : iv : '13 (d) (1) (Pl. 11, fig. 33).—Medullary tube and notochord both absent. On one side of the large yolk-plug is a slight protrusion: this is probably dorsal and represents the tail. The mesoderm in this tail is continued forwards into the body; ventral mesoderm is being differentiated.

There is no cœlom.

There are no suckers.

The anterior ectoderm is thickened, pitted and vacuolated, and there is a central fusion of yolk-granules. The gut is limited to the small archenteric cavity round the yolk-plug.

L. 1. 6 : iv : '13 (d) (2).—This is similar to the last. In the dorsal mesoderm there are indications of the differentiation of a median tract, the notochord.

L. 1. 6 : iv : '13 (e) (Pl. 11, fig. 34).—Like (d), except that the blastopore is closed, and the tail protuberance bilobed.

Of the two pits seen at the base of this tail one is quite superficial, a mere fold of ectoderm, while the other is a deep involution which passes in some way to end blindly. It is the proctodæum.

The vacuolated ectoderm is at the other (anterior) end.

L. 1. 6 : iv : '13 (f) (Pl. 11, fig. 35).—This is similar to (e) except that the rudiment of the tail is not bilobed. Of the two depressions at its base, one is a mere superficial folding, the other a deep passage leading into a cavity lying behind the yolk-mass, a rudimentary archenteron.

L. 1. 6 : iv : '13 (g) (1) Text-fig. 17).—This resembles (d) (2). There is an indication of the differentiation of the notochord—as a median band of smaller cells—from the dorsal mesoderm. There is a rudimentary archenteron opening under the dorsal lip of the blastopore.

L. 1. 6 : iv : '13 (g) (2).—Similar to the last and to (d) (1). There is no trace of a separation of the notochord.

8 : iv : '13.—The remainder were preserved.

One is undeveloped, 2 are normal in form with the tail longer than before, and 9 are monstrous. Among these tadpoles types (a), (b) and (e), (f) and (g) are represented each by one, types (c), (d), each by two specimens.

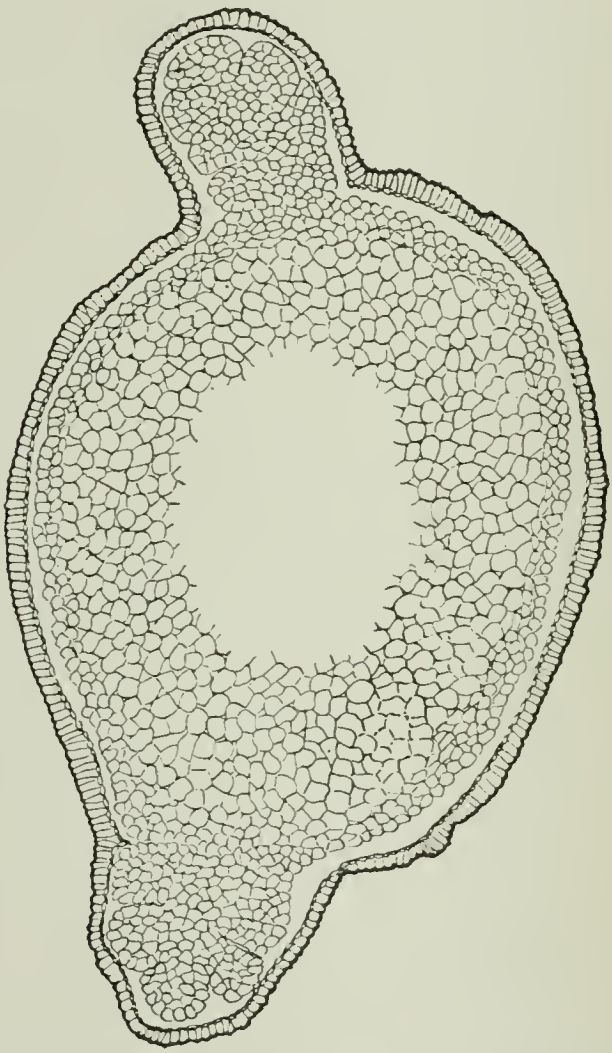
L. 1. 8 : iv : '13 (a) (Pl. 11, fig. 36).—The anterior ectoderm is very thick and pitted, and highly vacuolated.

There is no nervous system nor sense-organs. From the dorsal lip of

the blastopore a thick band of mesoderm stretches forwards; in front this becomes thinner. There is no trace of a notochord in it.

There is ventral mesoderm and the beginning of a pericardium.

TEXT-FIG. 18.



L. 1. 8 : iv : '13 (g). Both tails are cut. Signs of segmentation of the mesoderm in them. In the body mesoderm has been differentiated from the yolk-cells, in which is a central undivided mass of fused yolk-granules.

There is no sucker.

The blastopore is widely open; on one side is a deep involution, but except for this no gut. The yolk-plug is large. In the centre of the yolk there is a mass of fused granules.

L. 1. 8 : iv : '13 (b) (Pl. 11, fig. 37).—The head ectoderm is

vacuolated and thick. The fore-brain, with the optic vesicles and infundibulum, is present. The mid-brain is also present, and the hind-brain and spinal cord. All parts of the brain show cell-vacuolation.

Auditory vesicles, and ganglia, vagus ganglia, and ganglia of v and vii all formed, and neural crest. The suckers are present.

The notochord, normally vacuolated, extends to in front of the auditory vesicles. Both it and the spinal cord pass into the tail.

There is no stomodæum. The gill-slit outgrowths are solid.

The pericardium is large, but the heart rudiment is solid. The peritoneal cavity is still small. The pronephric tubules are developing, but are still without lumina and funnels. There is a liver diverticulum, but the lumen of the gut is obliterated.

There is fusion of yolk-granules in the yolk-mass, and a large yolk-plug.

L 1. 8 : iv : '13 (c) (1).—This resembles (a), but there is a deep involution underneath each lateral blastoporic lip. Otherwise there is no gut.

There are signs of the differentiation of a notochord.

There is no pericardium in the ventral mesoderm. The nervous system is absent, the longitudinal pleats looking like medullary folds being merely ectodermal ridges.

L. 1. 8 : iv : '13 (c) (2) (Pl. 11, fig. 38).—Like the last, but a rudimentary nervous system is present. A solid median mass of ectodermal cells with paired appendages represents the brain and ganglia. Imbedded in this are two small auditory vesicles. Behind this point a solid spinal cord runs back and enters one of the caudal swellings.

The dorsal mesoderm is a thick plate; it shows no signs of a notochord. The ventral mesoderm is present, but neither pericardium nor heart. The pronephric ridge is indicated on one side. The gut has no lumen. In the yolk-cells a central fusion of yolk-granules.

L. 1. 8 : iv : '13 (d) (1).—The anterior end can only be determined by the vacuolation of the ectoderm.

The segmentation cavity persists in front and dorsally. Below it is the mass of yolk-cells; its roof is formed of a band of mesoderm cells which passes back into the dorsal lip. This band stops before the front end is reached so that there the segmentation cavity is roofed only by ectoderm.

Ventral mesoderm is being differentiated. There is an open blastopore and persistent yolk-plug; the archenteric cavity is very small. There is neither notochord nor nervous system.

L. 1. 8 : iv : '13 (d) (2).—The segmentation cavity is obliterated and there is an archenteron, narrow, but longer than in the last. Otherwise the same.

L. 1. 8 : iv : '13 (e) (Pl. 11, fig. 39).—Body rounded, bearing a

short tail. No nervous system nor sense-organs. Dorsal mesoderm, but no notochord. Dorso-laterally the mesoderm is thickened and these two thickenings pass back into the tail. In each is a cavity (cœlom).

The ventral mesoderm is developed, but slight. There is an archenteron on the dorsal side with a postero-ventral extension. The latter is in communication with the exterior by an ectodermal involution (proctodæum).

The anterior ectoderm is vacuolated.

L. 1. 8 : iv : '13 (f) (Pl. 11, fig. 40).—The body consists of a solid mass of yolk-cells (in the centre of which is a region in which the granules are fused together), enclosed by a vacuolated ectoderm. On one side of the large yolk-plug is a tail. In this tail are paired segmented somites, with indications of myocœl. There is, however, no notochord. On the opposite side of the blastopore is a much shorter tail-like structure. The nervous system is completely absent. Dorsally and ventrally there are thin layers of mesoderm.

L. 1. 8 : iv : '13 (g) (Text-fig. 18, Pl. 11, fig. 41).—Body stumpy, with two tail-like structures, one on each side of the large blastopore.

Each of these tails contains mesoderm, which shows signs of being divided into pairs of somites. Otherwise this embryo resembles the last.

The pit at the anterior end is a mere infolding of the ectoderm.

2. Forty minutes after insemination, centrifuged for 10 minutes.

The central yellowish spot is surrounded by folds of the grey patch, the marginal white ring broader.

The first two furrows are normal in form, but retarded, not only on the controls, but slightly on L. 1.

2 : iv : '13.—The grey patch and yellowish spot are still visible. The animal hemisphere is as well segmented as in L. 1; the vegetative hemisphere is, however, divided into only a few large cells.

3 : iv : '13.—The grey patch is still present. The dorsal lip of the blastopore has not yet appeared.

4 : iv : '13.—There are indications of a blastoporic groove at the edge of the pigmented area.

6 : iv : '13.—The yolk is still exposed.

3. Seventy-five minutes after insemination, centrifuged for 16 minutes.

The folding of the grey patch round the yellowish spot is more marked; the spot appears to sink in. The marginal ring is more conspicuous and flecked with dark spots.

The first and second segmentation furrows may be normal in form, but there are many irregularities; the furrows of the third phase are also irregular, for instance, parallel to the first. These meridional furrows fail to reach the vegetative pole.

2 : iv : '13.—The grey patch and yellowish-white ring are still visible, but the foldings seem to have been smoothed out.

The animal hemisphere imperfectly segmented, the vegetative not at all.

3 : iv : '13.—No change.

4 : iv : '13.—There is still no sign of a blastopore.

4. Seventy-five minutes after insemination, centrifuged for 25 minutes.

The marginal zone becomes broader still.

Segmentation as in 3, or even more irregular. The yellowish spot may become cut off as a separate "cell," the folds being incorporated in the divisions or else entirely smoothed out.

2 : iv : '13.—The white ring is still visible.

Segmented in the animal hemisphere.

3 : iv : '13.—As 3.

4 : iv : '13.—There is no sign of a blastopore.

6 : iv : '13.—The white ring is now very faint. No other change (Pl. 9, fig. 14).

M.

Centrifuged 2 : iv : '13 one hour after insemination at speed II on the water-driven machine.

1. For 10 minutes.

A circular grey patch appears round the animal pole, containing sometimes a yellowish spot; it is surrounded by a paler border (Pl. 7, fig. 8*a*).

The first and second furrows are meridional as normally, but sometimes either of these divisions is unequal. They appear later than in the controls.

The furrows of the third phase are abnormal in being meridional. All these furrows reach the vegetative pole.

3 : iv : '13.—Segmentation is completed; the cells of the vegetative hemisphere are rather large.

The grey patch has disappeared.

4 : iv : '13.—There is a blastopore, three quarters of a circle or circular; it is rather large.

6 : iv : '13.—The medullary folds are closing (when they have been formed). The blastopore is still open in some cases.

Six embryos were preserved at this stage, one each of types (a) and (b), two each of types (c) and (d).

M. 1. 6 : iv : '13 (a).—Rounded body, yolk-plug still exposed. A slight back-growth of the body on the dorsal side of the blastopore is the beginning of a tail. The archenteron is short and does not extend in front of the middle of the body.

Fusion of yolk-granules occurs.

There is no medullary plate. The dorsal mesoderm is hardly differentiated from the roof of the archenteron. There is no distinct notochord; ventral mesoderm is present.

Anteriorly the ectoderm is thickened but not so remarkably as in later stages. It is slightly pitted.

M. 1. 6 : iv : '13 (b).—The anterior ectoderm is vacuolated. There are no suckers.

Neither olfactory pits nor eyes are present. The auditory vesicles are curved plates, slightly detached from the ectoderm.

A mass of pigmented cells in front probably represents the fore- and mid-brains. Similar lateral masses would be the ganglia of this region, possibly also the optic vesicles. The median mass is continued into a fold, and this passes behind into a solid cord which presently enlarges. The lumen of the hind-brain appears in this. The lumen is dorsally situated. The hind-brain is continued in turn into the spinal cord, in which the lumen is also excentrically dorsal; posteriorly, however, it is in the normal position.

The neural crests come fairly far down but do not meet below the cord.

The vertebral plates are united across the middle line by horizontally elongated cells. There is no notochord.

A pronephric ridge is present and there are indications of a splanchnocœl in the lateral plate.

Ventral mesoderm is present but there is as yet no pericardium.

The enteron is narrow but extends far forwards.

There is a central fusion of yolk-granules.

There is a proctodæum, and a streak of pigment-cells indicates the neurenteric passage.

The yolk-plug has been withdrawn.

M. 1. 6 : iv : '13 (c).—As (a), but there is no back-growth above the yolk-plug, and the archenteron is shorter.

M. 1. 6 : iv : '13 (d).—As (b), but the medullary tube is smaller and the lumen minute.

There is a yolk-plug.

8 : iv : '13.—A tail is developed in most. The head is deficient.

Seven embryos were preserved, two each of types (a) and (c) and one each of types (b), (d) and (e).

M. 1. 8 : iv : '13 (a).—Anteriorly the ectoderm is vacuolated. Here there is a large cavity bounded by a layer of flattened cells. The cavity is the persistent segmentation cavity, the lining cells mesodermal elements which have been differentiated from the yolk-cells and passed into the cavity, while the yolk-cells have remained in their original position.

Posteriorly the mesoderm cells pass into the main mass of yolk-cells in which is the enteron.

There is a central mass of fused yolk-granules.

Dorsal mesoderm is present and differentiated into vertebral and lateral plates. A median notochordal tract is distinguishable from the vertebral plate in places.

Pronephric tubules are in course of formation.

Ventral mesoderm not well developed.

There is no nervous system. What appears from the outside to be such is a median fold of ectoderm enclosing some mesodermal elements. Proctodæum large but not open into enteron.

M. 1. 8 : iv : '13 (b) (Pl. 11, fig. 42).—Anterior ectoderm vacuolated, enclosing a persistent segmentation cavity. In front this is bounded by the ectoderm alone, but further back a thin layer of cells appears lying inside the ectoderm. This layer is continued into the mass of yolk-cells behind. In the latter a central mass of fused granules.

The dorsal mesoderm passes back into the tail, and is there segmented. There is no notochord. Ventrally the mesoderm is poorly developed.

The cavity of the enteron is small and short. The proctodæum opens into it.

There is no nervous system.

M. 1. 8 : iv : '13 (c) (Pl. 11, fig. 43).—As in (a) and (c) there is anteriorly a persistent segmentation cavity. It is not, however, lined in whole or in part by a sheet of mesoderm, but merely includes a certain number of mesodermal cells.

The yolk plug is exposed, and there is a short archenteron opening by the blastopore.

The tail springs from a thick mass of mesoderm lying beneath the lip of the blastopore on one side; there is a similar mass of mesoderm on the opposite side, and the two become confluent in front.

Correct orientation of this embryo is very difficult as there is neither nervous system nor notochord, but assuming that the mesodermal thickenings are lateral the tail is either right or left. The side on which the two masses meet may then be dorsal.

M. 1. 8 : iv : '13 (d).—The anterior vacuolated ectoderm encloses a solid mass of yolk-cells, with peripheral mesoderm. The segmentation cavity does not persist.

The mesoderm becomes concentrated posteriorly into three masses which project wedge-like into the yolk-cells. At the hinder end these three pass into the blastoporic lip, where they become continuous with small cells on the surface of the yolk-plug. Enteron, nervous system and notochord are all absent.

M. 1. 8 : iv : '13 (e).—The anterior vacuolated ectoderm encloses a

solid mass of yolk-cells. Peripherally mesoderm is differentiated. Posteriorly there is a short tail springing from what appears to be the dorsal side, since the archenteron is on this and the proctodæum on the opposite side.

The proctodæum ends blindly.

The tail contains mesoderm which springs from a dorsal median, partly also from a lateral concentration of mesoderm.

Nervous system and notochord both absent.

2. For 20 minutes.

The grey patch is larger. A grey ring appears outside the white ring.

Segmentation as in 1 (Pl. 7, fig. 8, *b*).

3 : iv : '13.—The grey patch is still to be seen.

The vegetative hemisphere is imperfectly segmented.

4 : iv : '13.—No blastopore has appeared.

3. For 30 minutes.

There is a yellowish central spot inside the grey patch.

Segmentation more irregular. The second furrow, for instance, may be parallel to the first, and the divisions do not reach the vegetative pole.

3 : iv : '13.—The grey patch is still visible. The vegetative hemisphere is not segmented.

4 : iv : '13.—No blastopore has yet been developed.

N.

Centrifuged 2 : iv : '13 on the water-driven machine one and a half hours after insemination at speed IV.

1. For 10 minutes.

The circular grey patch contains a yellowish spot surrounded by folds. Round it is a yellowish-white margin.

By the time segmentation has begun the folds have disappeared and the central spot is not very distinct.

The first furrow may be normal but is not always so. The second also may be meridional, but is not so always. Later furrows are irregular, sometimes meridional, sometimes circular—that is, circumscribing a small area in the animal hemisphere. The meridional furrows do not reach the vegetative pole.

3 : iv : '13.—The animal hemisphere alone is segmented, and that incompletely.

4 : iv : '13.—No development has occurred.

2. For 20 minutes.

There is a grey ring outside the white margin.

The first two furrows may be meridional but are not necessarily so. They do not reach the vegetative pole. Later furrows are very irregular.

3 : iv : '13.—As 1.

4 : iv : '13.—No development.

3. For 30 minutes.

The grey patch is surrounded by a groove. Outside this is first a white, then a grey ring. Segmentation is very seldom regular, even in the earliest stages. Divisions are often parallel to a meridian, and circular furrows are common. The vegetative hemisphere is not segmented.

3 : iv : '13. } As 2.
4 : iv : '13. }

O.

Centrifuged 3 : iv : '13 on the water-driven machine at speed IV.

All these eggs were preserved in formol immediately or in early cleavage stages.

1. One hour after insemination, centrifuged for 5 minutes (Pls. 7, 8, 10, figs. 1, 9, 16).

A circular grey patch appears round the animal pole; in it are radial dark striæ converging towards a central yellowish spot. The patch is separated by a groove from the pigmented area.

The patch is sometimes grooved or folded. The first and second furrows may be meridional, but are often parallel to a meridian. Later furrows are very irregular. Some, but not all of the meridional furrows reach the vegetative pole.

2. One hour after insemination, centrifuged for 10 minutes (Pl. 7, fig. 2).

The central spot is slightly depressed and surrounded by folds of the grey patch. The patch is marked by radial striæ, and surrounded by a groove. It has a narrow, faint whitish border.

Segmentation as in 1.

3. One hour after insemination, centrifuged for 15 minutes (Pls. 7, 8, 10, figs. 3, 10, 17).

The central spot is sinking in and being covered over by the folds. The radial striæ are still present. The white border is broader. Outside the groove is a grey ring.

Segmentation is more irregular than in 2. The furrows hardly reach the vegetative pole.

4. One and a quarter hours after insemination, centrifuged for 20 minutes (Pls. 7 and 8, figs. 4, 11).

The central spot is no longer visible, the folds having grown over it. The grey patch is still radially striated. External to the marginal groove is a second white ring, just above the grey ring and derived from it.

The first furrow may reach the vegetative pole, the others do not. Circular furrows are seen.

5. One and a quarter hours after insemination, centrifuged for 25 minutes.

The folds have nearly met and fused. Internal to the white border of the grey patch is a yellowish ring.

The furrows, when meridional, do not pass beyond the pigmented area (Pl. 7, fig. 5).

6. One and a half hours after insemination, centrifuged for 30 minutes (Pls. 7, 8, and 10, figs. 6, 12, 18).

The grey patch is now homogeneous, the radial striæ having disappeared. It is smaller, and immediately surrounded by a groove.

Outside this groove is a wide white ring which appears to be due to the confluence of the two white rings of the earlier stage.

Then comes the grey ring subdivided into three zones, of which the middle is darker.

Segmentation very irregular. The first furrow may begin at the side instead of at the animal pole. Circular furrows are found.

(3) The Effect produced by the Centrifuge on the Structure, Segmentation, and Development of the Egg.

From the details of the experiments that have been given in the preceding section it will be clear that the effect produced by the centrifuge upon the structure of the ovum, and upon its segmentation and development, varies with the degree of acceleration and the length of exposure.

A strict comparison is, of course, only possible between eggs of the same batch, centrifuged on the same machine, at the same time, at different speeds and exposures. Obviously, however, these conditions cannot always be observed, and then, owing, as already explained, to diurnal, if not hourly inconstancies in the water pressure, as well as to variations in the eggs themselves, it may well happen that on some occasion an exposure to a higher will not effect a greater change, will effect, perhaps, even a less change than an exposure of the same length to a lower acceleration.

Fortunately, in my series of experiments, this has not occurred, except, perhaps, once.

The accompanying table gives a résumé of the experiments performed with the water-driven centrifuge:

Speed.	Exposure in minutes. 5.	10.	15.	20.	25.	30.
I	—	H. 3	—	—	—	—
	—	J. 1	—	J. 2	—	—
II	—	H. 2	—	—	—	—
	—	K. 1	—	K. 2	—	—
	—	M. 1	—	M. 2	—	M. 3
III	—	H. 1	—	—	—	—
	L. 1	L. 2	L. 3	L. 4	—	—
IV	—	I. 1	—	—	—	I. 2
	—	N. 1	—	N. 2	—	N. 3
	O. 1	O. 2	O. 3	O. 4	O. 5	O. 6

On referring back to the preceding section it will be found that in nearly all cases the same acceleration and exposure produce the same, or nearly the same, effect upon the egg.

Thus in H. 3 and J. 1 there is a grey patch only, without a markedly white border. In H. 2, K. 1 and M. 1 the patch is surrounded by a lighter ring. In K. 2 and M. 2 the patch is folded round a central lighter spot, but in M. 2 there is an additional grey ring, which in K. 2 is absent. In H. 1 and L. 2 the patch is deeply infolded. In I. 1, N. 1, and O. 2 there is a central yellowish spot in the grey patch, and the latter has a lighter border. In N. 2 and O. 4 the grey patch is deeply infolded, the white border marked, while in I. 4, N. 3, and O. 6 the grey patch is surrounded by a broad white border, and this by a composite grey ring. When the same speed is employed the effect is always increased by prolonging the exposure. Conversely, when the exposure is constant the effect ought to vary with the acceleration, but this has not proved to be the case invariably. Thus the change in J. 2 is less than in K. 2, and less in K. 2 than in L. 4, and similarly in the series J. 1, K. 1, L. 2, and, again, less in M. 3 than in O. 6, but H. 1 and L. 2 are very like O. 4, L. 4 like O. 6, so that here the case is realised of a lower acceleration having caused a greater alteration than a higher in two different lots of eggs, the exposure being the same.

The same discrepancy appears in the subsequent develop-

ment, the embryos of I. 1 being as well if not better developed than those of L. 1 and M. 1 (which are much alike). Development only occurs in the series J. 1 and J. 2, H. 3, I. 1, K. 1, L. 1, M. 1, and G. 1 and G. 2. The ova of the series H. 2 did not develop though it would have been expected that they should. O. 1, N. 1 and O. 2 would very possibly have developed if they had been kept.

Of these J. 1 and J. 2 develop best, then H. 3, then G. 1, then G. 2, then I. 1, L. 1 and M. 1. The position of the embryos of K. 1 in the list is uncertain since they were unfortunately not preserved. There is, however, sufficient evidence that in the main the capacity to develop is progressively decreased as the acceleration is raised and the exposure prolonged. The experiments of the G series can only be assigned a position amongst the others by the extent to which the egg-structure and development are altered. In G. 1 and 2 there is a simple grey patch, and development is much better than in any except J. 1 and J. 2. They probably lie very near H. 3. G. 3 and G. 4 have the ring round the grey patch and fail to develop.

We may now proceed to discuss in order the effects which the operation produces upon the structure of the egg, its segmentation and its development.

a. The Effect Produced upon the Egg-structure.

The Structure of the Normal Egg.— As is well known, the spherical egg of the frog has a radially symmetrical structure about an axis, which axis has unlike poles. The polarity is determined first by the distribution of the superficial pigment, which only occupies about two thirds of the egg-surface, and second, by the disposition of the plasma (protoplasm) and yolk, the plasma being mainly, though not exclusively, situated in the pigmented region, the yolk-granules larger and more abundant in the unpigmented region, though found, of course, in the plasmatic portion as well. The line drawn through the centre (at the surface) of the pigmented

and plasmatic region, the centre of the egg itself, and the centre (at the surface) of the unpigmented yolky region is the axis, and its unlike plasmatic and yolky poles respectively the animal and vegetative. Internally there is also diffuse pigment, aggregated a little more intensely in the axis and in the animal hemisphere. The nucleus of the full-grown but immature oocyte lies, of course, axially, but excentrically, in the animal hemisphere, and it is in the same place that the pronuclei meet in fertilisation. All this is, of course, a common place of embryology, but the distribution of certain other substances in the ovum has not, as far as I am aware, ever been described. I allude to the fat (including lecithin, which, as I shall have occasion to show later, is present) and the glycogen. The fat appears to be uniformly distributed in the shape of small globules, variable in size, lying in the plasma between the yolk. These globules are easily demonstrated in sections of eggs, preserved in formalin and frozen, by means of the fat stain Sudan III. The yolk-granules remain uncoloured by the dye.

In respect of the glycogen the egg is, however, polarised, for this substance, present in the form of small spherules, is more abundant in the plasma of the animal than in that of the vegetative region, the spherules being larger and more numerous in the former, while in the latter they are excessively minute and very scanty (Pl. 10, fig. 15).

The glycogen, therefore, like the plasma in which it is embedded, gradually decreases in concentration from the animal to the vegetative pole, while the concentration of the yolk increases in the opposite direction.[1]

This polar structure is seriously affected when the egg is centrifuged, in my experiments in its axis. Roughly speaking what happens is that the lighter fat comes to the centripetal, that is, the animal pole; the next lightest, the plasma with the

[1] The distribution of glycogen in the course of development has not yet been worked out. I have only observed that in tadpoles in which the operculum is closing this substance is found only in the myotomes, in the tubules and duct of the pronephros and in the roof of the medulla.

glycogen, forms a layer next to the fat; while the heavy yolk and pigment are driven to the centrifugal, the vegetative pole. Hence the various zones or strata into which these eggs become divided. The somewhat complex details of the changes are most readily made out in a series of eggs centrifuged at the same acceleration with successively longer exposures, as, for example, the series O. The acceleration was here considerable: the exposures were 5, 10, 15, 20, 25 and 30 minutes. The ova were preserved in formol; from this material good sections are obtainable, either by the paraffin method, or after freezing. The latter are necessary for the study of the fat.

In O. 1 (Pl. 7, fig. 1, *a*, *b*), there is developed round the animal pole a circular grey patch radially striated with dark lines converging towards a central yellowish spot. The patch is separated by a groove from the surrounding pigment. The radial striæ are the lines along which the pigment is streaming away from the centripetal pole, while the lighter fat and plasma is moving in the opposite direction.

The yellowish spot may be excentric, and the grey patch may be folded.

A meridional section shows, beginning at the animal pole (Pls. 8 and 10, figs. 9, 16), (1) a superficial layer of rather finely vacuolated plasma, staining violet with hæmatoxylin. In it there is an occasional yolk-granule. Some of the original pigment remains in this layer, being disposed in (α) a denser sheet at the surface, (β) more diffusely below.

This layer is the grey patch seen from the surface.

(2) A more coarsely vacuolated layer of the same violet staining plasmatic substance. In it is some pigment and a few yolk-granules. At the level of this layer is one enormous vacuole (*v.*) which presses out layer 1 on the centripetal side. The floor of this vacuole is formed of the next layer. Both the large vacuole and the smaller vacuoles of layers 1 and 2 contain fat, staining vividly with Sudan III, and it is the large vacuole which is seen from the outside, shining through the first layer, as the yellowish spot.

(3) A layer of a violet-staining plasmatic substance which appears to have an alveolar structure (though this may be the effect of one of the reagents used). This layer is composite, there being denser sheets with a good deal of pigment running through lighter patches which contain little pigment. Fat vacuoles, singly and in groups, occur in this layer, and a good deal of yolk remains in it.

Neither the second nor the third layer comes to the surface, the first layer being here co-terminous with the fourth.

(4) The yolk and pigment.

Immediately below layer 3 is a sheet of pigment from which dense streamers depend into the yolk below. The original sheet of superficial pigment remains at the surface.

(5) The unpigmented region round the vegetative pole forms a fifth layer, but this, of course, was not produced by the centrifuge.

A good deal of fat and plasma remain still in the yolk.

In O. 2 the central spot is slightly depressed, and a white border is beginning to appear around the edge of the grey patch (Pl. 7, fig. 2, *a*, *b*).

Sections show the following layers:

(1) With the same characters as in O. 1. Below it a large fat-vacuole.

(2) With the same characters as O. 1, but with still less pigment, hence the appearance of a white border round the grey patch. This layer has probably been reinforced by some plasma from the third layer seen in O. 1 from which the yolk-granules have been drawn away.

(3) A broad band with numerous small yolk-granules—those found in the third layer of O. 1—and some vacuolated plasma. The latter has presumably just come up from the yolk below. This layer lies immediately centrifugal to the layer of pigment.

(4) The rest of the yolk with large granules. The superficial pigment is unaltered. As layer—

(5) may be distinguished the original unpigmented area.

In O. 3 the central spot begins to sink in below the sur-

rounding folds of the grey patch, while the white border to the latter is more distinct. Immediately outside this there is in the pigmented area a grey ring (Pl. 7, fig. 3, *a*).

The section shows the following layers (Pls. 8 and 10, figs. 10, 17) :

(1) The coarsely vacuolated pigmented layer in which the large vacuoles are embedded. This is the grey patch which is folded. The folds appear to arise by the accumulation of large globules or vacuoles of fat. As these are forced more and more centripetally the folds which are caused by them pass over the central depressed portion of the grey patch, which itself appears yellow owing to the accumulation of fat there.

In some of the vacuoles of this layer there is a coagulated fluid, which is stained pink with eosin. This appears to be a protein distinct from the material of which the walls of the vacuoles are composed. Whether the same vacuoles also contain fat is not certain ; in that case this might be a protein material accompanying the fat—the thin envelope, perhaps, of the fatty globules. On the other hand, the eosinophilous material may be normally associated with the other proteins of the plasma, and only dissociated from it by a certain degree of centrifuging. We shall see later that there is evidence for the existence of more than one kind of protein in the plasma.

(2) A layer which appears homogeneous under a low, finely alveolar under a high power. It stains violet with hæmatoxylin. In it are a few fat-vacuoles, but it contains no yolk-granules. Pigment is scattered through it, and the remains of the superficial pigment is seen at its external surface. It is the white border of the grey patch. This is identical with layer 2 in O. 2, but is thicker, and has lost nearly all its fat.

(3) Below this stretches a thin sheet of pigment, and immediately below this a broad vacuolated layer. The walls of the vacuoles are formed of plasma—staining with hæmatoxylin—with yolk-granules embedded in it; the cavities of the vacuoles are occupied by fat. Pigment is scattered through this layer, but sparsely, and at the external surface the

denseness of the original pigment sheet is much diminished. Externally this appears as the grey ring.

This layer is evidently derived from layer 3 of O. 2. What has happened is that more fat-globules have been driven centripetally and accumulated here in vacuoles underneath the dense plasma of layer 2, through which at present they are unable to pass.

In this layer are found local accumulations of deeply staining (violet) plasma. The plasma is, of course, being driven centripetally. There are also vacuoles containing eosinophilous coagulum.

(4) The pigmented region of the yolk. The lower edge of the pigment sheet is driven inwards.

(5) The pigment-free yolk.

O. 4. (Pl. 7, fig. 4, *a*, *b*). The folds of the grey patch have nearly met. A second white ring, derived from the grey ring, lies just external to the white ring of the grey patch.

The section (Pl. 8, fig. 11) reveals the same layers as in O. 3. The third layer—of fatty vacuoles with yolk-granules in the walls—is emancipating itself from the sheet of pigment, and sending streamers into the plasmatic layer (layer 2) above. The yolk-granules are evidently adherent—for the moment—to the fat-globules, and caught up in the centripetal movement. This gives the white division of the grey ring. Layer 2 is now practically devoid of pigment. The other layers are as in O. 3.

O. 5 (Pl. 7, fig. 5, *a*) presents very little change beyond the close approximation of the lips of the folds of the grey patch, but in—

O. 6 great alterations have occurred.

The grey patch—no longer radially striated—is immediately surrounded by a deep groove. Outside this lies a very broad white ring, and then a grey ring, compounded of three zones, the middle of which is darker than the others (Pl. 7, fig. 6, *a*).

Sections (Pls. 8 and 10, figs. 12, 18) show that (1) the grey

patch is composed of the same coarsely vacuolated pigmented material as before, and encloses a large fat-vacuole; (2) outside the groove which bounds this patch is another layer of vacuolated material, but this includes but little pigment. The vacuoles contain fat.

(3) There follows the finely alveolar plasmatic layer, almost devoid of pigment, with a few yolk-granules, and here and there a fat-vacuole. With the transitional condition O. 4 before us it seems easy to understand what has happened. The fat of layer 3, which was there beginning to penetrate the plasmatic layer 2, has completely passed through the latter and given rise to the present layer 2—a layer which, as we should expect, contains but little pigment. In so doing it has shaken off the yolk-granules, which have passed back in the opposite direction, although a few remain entangled in the third layer. The second and third layers then form together the broad white wing. In this process the original groove external to the white ring (layer 2 of O. 3 and O. 4) disappears, while a fresh groove is formed round that portion of the fatty layer in which a considerable quantity of pigment is still entangled, namely, in the grey patch.

(4) Underneath the homogeneous plasma layer is a sheet —the uppermost sheet of the yolk—from which a good deal of the pigment has disappeared. This is the grey ring.

In it a finely vacuolated layer can be distinguished above from a layer which is not so vacuolated. The former is the paler upper zone of the grey ring, and is due to a fresh agglomeration of fat-globules in vacuoles below the plasma layer. In other words, the process seen in O. 4 is about to be repeated. It must be remembered that there is still a good deal of fat left in the yolk. With Sudan III the yolk stains a faint orange, and proper examination reveals the fat-globules between the yolk-granules (Pl. 10, fig. 18 *c*).

I have not succeeded in finding in the sections the lower pale zone of the grey ring.

The other layers are as before.

We can now form some idea of the changes that take

place when the egg is centrifuged at this high acceleration for progressively longer periods.

First the pigment and yolk are driven centrifugally while the fat and plasma are urged in the reverse direction. The fat is, however, lighter than the plasma, so that the former occupies the most centripetal position. At the same time the globules become agglomerated into larger masses, some of which are enormous; a certain amount of pigment remains obstinately adherent to the fat. Hence the grey patch and yellow spot. The plasma forms a layer next to this, and gradually rids itself of fat and yolk, in opposite directions. When it is free from pigment it appears as the white border. At the same time the pigment is spread out in a third layer, which sends streamers into the yolk below. This fact, coupled with the frequent mottling of the original unpigmented area, suggests that the pigment is perhaps heavier than the yolk.

The supply of fat and plasma in the vegetative region is, however, by no means yet exhausted, and a fresh accumulation is soon spread out beneath the barrier, at present impenetrable, of the plasma layer. But eventually this gives way, the fat passes through, dragging at first some yolk-granules with it, but these are quickly discarded and driven back. The condition seen in O. 6 is thus reached, though this is by no means the final stage, since there are evident preparations for a fresh conglomeration of fat. What the end would be is clear enough. The fat—with adherent pigment and plasma—would be centripetally disposed, the yolk and pigment centrifugally, while the plasma, including, I may perhaps now say, the glycogen, would lie between. A discussion of the chemistry of the components of these layers must, however, be reserved for a later chapter.

The same kind of effect is produced with a smaller acceleration, but, as a rule, the white ring appears before the central spot—as, for example, in series M (Pl. 7, fig. 8). Probably considerable force is required to make the fat-globules cohere in one or more large masses.

A section of K. 1 (Pl. 9, fig. 13) shows the grey patch as a coarsely vacuolated layer, with pigment, the pale border as a plasma layer, with some pigment, and below this a sheet of pigment and the yolk.

When the acceleration is smaller still, as in series J (Pl. 7, fig. 7), the only alteration revealed by the sections is an immigration of pigment round the animal pole. This causes the faint grey patch seen in these eggs.

As a rule the various zones and rings become somewhat confused, and the folds of the grey patch disappear after a short interval; but a grey or blotched patch usually persists for a considerable time, and may often be seen at the anterior end of the embryo.

There is, in fact, a slight re-diffusion of the disarranged materials through one another, but the normal arrangement is never regained—not at least in eggs centrifuged so soon before segmentation.

b. The Effect produced upon the Segmentation of the Egg.

With the lower acceleration the segmentation of the egg is normal except for a slight retardation, as for example in L. 1, L. 2, M. 1 and M. 2 (Pl. 7, fig. 8), but when greater force is applied irregularities appear (Pl. 7, figs. 1 *c*, 2 *c*, *d*, 3 *b*, 4 *c*, *d*, 5 *b*, 6 *b*, *c*). Even though the first two furrows are meridional, those of the third phase may be meridional instead of latitudinal (as in L. 4) or parallel to the first. The first and second are often parallel to a meridian, and with greater accelerations the normal sequence is almost entirely abandoned. The first furrow may begin at the side instead of at the pole (O. 6), and circular furrows, cutting off a small region of the animal hemisphere completely or incompletely, are frequently observed (O. 4, O. 6, L. 4). Many or all of the meridional furrows fail to reach the vegetative pole, and as a result the segmentation becomes more or less meroblastic; in extreme cases, indeed,

it ends in the formation of a blastoderm resting upon an unsegmented yolk, as Oscar Hertwig pointed out; as mentioned by the same author the nuclei in the yolk become enlarged, irregular in shape and highly chromatic, so resembling the yolk nuclei of Elasmobranchs and Teleostei, bodies which are concerned in the elaboration of the yolk alone, and do not play any part in the formation of the embryo.

A section (Pl. 9, fig. 14) through one of these eggs (L. 4, 6 : iv : '13) shows that though five days have elapsed since the operation no differentiation has occurred. The egg has merely continued to segment slowly.

Some of the strata can still be recognised.

Round the animal pole is the grey patch, lightly pigmented and vacuolated. This region alone is properly segmented, and even here it is not possible to distinguish cell-boundaries between the nuclei of the deeper layers. There are three or four layers of nuclei in all.

The grey patch passes into the remains of the plasmatic layer, but this contains now masses of yolk-granules and a good deal of pigment, both of which have evidently returned from the inferior position to which they were driven. In this layer are numerous large vacuoles, some clear (these evidently contained fat), and some filled with a coagulated liquid which stains with the plasma dye (picro-indigo-carmine). The fat-globules are presumably due to the breaking up of the larger vacuoles of the centrifuged egg—again a return of a substance towards its original position. This layer contains nuclei, many of which are homogeneous and highly chromatic, while others are very large and irregularly amœboid.

Below is the yolk, in the upper (centripetal) region of which are a few nuclei, of a large but not excessive size.

c. The Effect produced upon the Development of the Embryo.

We have at our disposal embryos and larvæ obtained from the series J, H. 3, G. 1, G. 2, I. 1, L. 1 and M. 1.

In the series J the acceleration was small and the tadpoles were normal.

In the series G. 1 a larger acceleration was (probably) employed, and out of 53 tadpoles 12 were abnormal. These tadpoles were twelve days old when preserved. The H. 3 ova were subjected to about the same force as those of G. 1, but the embryos were killed at an earlier stage when five days old. In G. 2 the same force was used as in G. 1 but the exposure was longer: out of 43 larvæ (killed at 12 days) 15 were abnormal. The force employed and the effect produced in the remaining series were greater, the degree of abnormality being progressively larger in I. 1 (10 abnormal out of 13), L. 1 (17 abnormal out of 21), and M. 1 (all abnormal). The numbers are, however, too small to be genuinely significant, and all the embryos of these three series may be placed in one class. Those of I. 1 were preserved on the third and fourth days, those of L. 1 on the fifth and seventh days, and those of M. 1 on the fourth and sixth days after the operation.

The available material should, therefore, provide an opportunity for the study of the genesis of these aberrations of development.

The distortion of development produced by the centrifuge is of a very striking kind, and is, moreover, one which cannot be induced as far as I am aware by any other method. It consists essentially of, first, a disturbance at the anterior end, which may manifest itself merely by a vacuolation of the ectoderm and of the nervous system and other structures in that region, but more usually takes the form of a total disintegration of the front part of the head: the olfactory pits, the fore-brain and eyes, and the mid-brain, the skull and the mouth, all disappear as such, and the nervous system begins in the region of the medulla. Secondly, the yolk is affected. The only sign of any derangement may be a tract of undivided yolk in which the granules have become fused into one mass, but the closure of the blastopore is often prevented, or at least delayed, and there is a more or less persistent yolk-plug. When it is remembered that the yolk-plug is derived from

material situated in the vegetative region of the egg while the head end of the embryo is developed near the animal pole, the significance of the relation between these malformations and the structural derangement produced by the centrifuge along the axis of the ovum will be sufficiently obvious. While these abnormalities are proceeding in the head and round the blastopore the middle region may be developing normally, such structures as the auditory vesicles, medulla and spinal cord, pharynx and gill-clefts, branchial skeleton (in part), lungs, heart and blood-vessels, alimentary canal, pronephros, germ-cells and tail may all be of ordinary appearance. (The tail, I may remind the reader, though, of course, posterior, is developed from the lateral lips of the blastopore—that is, from material placed originally in the equatorial region of the egg.) There is, however, one curious malformation in this region, which may best be described as a fusion of paired structures in the middle line. It is seen in the abnormal arrangement of neuroblasts in the medulla and spinal cord, in the fusion below the spinal cord of the paired spinal ganglia, sometimes of the posterior cranial ganglia too, and in the fusion below this, again, of the mesodermal somites, or rather of the myotomes. The last leads to the obliteration of the notochord. Exceptionally the auditory vesicles may unite above the medulla. All these changes occur when the ova have been subjected to a moderate degree of force (as in the G. series): when a greater force is employed, the derangement is more serious; the whole of the nervous system and the organs of the middle region of the body may disappear, leaving an embryo with perhaps a very short archenteron and an undifferentiated mesoderm. A longer or shorter tail may, however, grow out; sometimes it is double, and the mesoderm contained in it may show traces of segmentation.

These monsters occur mainly in the L. 1 and M. 1 series; that they are not merely retarded embryos of an early stage, which might possibly have developed further, is indicated by their age—four to seven days—and by the extreme irregularity of their appearance.

The Vacuolation of the Anterior Ectoderm (Pl. 12, fig. 44).

This alteration occurs at the front end, extending back some little way on both dorsal and ventral sides, but never reaches quite to the posterior end though the ectoderm is here often folded. Where the centrifuging has been more severe it is more extensive.

In slight cases the ectoderm remains two-layered and only the epidermal layer is affected (as in G. 1, 8 : iv : '13 [a and b]), but after a more violent operation the ectoderm becomes thickened by an increase in the number of layers and at the same same time folded and pitted and the cells in all layers are affected. At the same time the vacuoles are larger (as in G. 2, and the embryos of the I., L. and M. series). There can be no doubt that in the fresh condition these vacuoles were full of the fat forced by the centrifuge to the animal pole. Other structures at the anterior end may be similarly vacuolated ; the olfactory pits (G. 1, 8 : iv : '13 [a]), the brain (G. 1, 8 : iv : '13 [a], I. 1, 3 : iv : '13 [a, b and c]), the optic vesicles (I. 1, 3 : iv : '13 [a and c]), the auditory vesicles (G. 2, 8 : iv : '13 [a]), the ganglia of cranial nerves, and mesoderm cells (G. 1, 8 : iv : '13 [b], G. 2, 8 : iv : '13 [a]).

The Degeneration of the Front Part of the Head.

In G. 1, 8 : iv : '13 (a) (Pl. 11, fig. 19) the brain and skull are normal and there is a mouth. The only abnormality, indeed, to be noticed is the absence of a lens. The optic cup has a narrow mouth and is some little distance from the ectoderm (Text-fig. 1).

In such early stages as I. 1, 3 : iv : '13 (a, b, and c), H. 3, 2 : iv : '13 (a) (Pl. 11, figs. 24, 26, 27), both fore-brain and mid-brain are present and apparently normal; while normal olfactory pits are found in H. 3, 2 : iv : '13 (a), I. 1, 4 : iv : '13 (a and b), and optic vesicles in H. 3, 2 : iv : '13 (a), I. 1, 3 : iv : '13 (b).

As a rule, however, these structures, together with the front part of the hind-brain, suffer disintegration by the time such stages as G. 1, 8 : iv : '13 (b), G. 2, 8 : iv : '13 (a, b, c, e, and g) (Pl. 11, figs. 20, 21, 23) are reached. All that remains of them is then a mass of pigmented vacuolated cells lying in the front of the head (Text-fig. 2, *a*), some of which have the large pale nuclei characteristic of neuroblasts (Pl. 12, fig. 45), and from some of which nerve-fibres proceed. Mingled with the cells is a débris of cell and nuclear fragments (the latter having undergone chromatolytic degeneration into deeply staining spherules), yolk- and pigment-granules.

The ganglia of this region (v and vii) often preserve their individuality more or less completely (G. 1, 8 : iv : '13 [b], G. 2, 8 : iv : '13 [a, c, g]); they appear as groups of vacuolated pigmented neuroblasts, and the appropriate nerves can often be traced (Pl. 12, fig. 46). In one case (G. 2, 8 : iv : '13 [a]) the olfactory sacs remain as two vacuolated fibrous masses, still retaining their connection with the ectoderm. No trace of the eyes is found in these embryos.

At an earlier stage the rudiments of these organs were laid down; their degeneration seems to set in almost at once. Thus in H. 3, 2 : iv : '13 (b), L. 1, 6 : iv : '13 (a), the front part of the brain is a solid wedge of cells (the mid-brain region in the last-mentioned is an open groove), in L. 1, 6 : iv : '13 (b) the brain is very small, the olfactory pits are very shallow in H. 3, 2 : iv : '13 (b), I. 1, 4 : iv : '13 (c), and the optic vesicles thick-walled (I. 1, 3 : iv : '13 [a]) or much reduced (L. 1, 6 : iv : '13 [a], I. 1, 4 : iv : 13 [c]) (Text-fig. 3). Associated with débris of the nervous system and sense organs are of course mesodermal elements, such as wandering connective tissue cells and chromatophores. Mesodermal cells may suffer vacuolation (G. 1, 8 : iv : '13 [b] (Pl. 12, fig. 45 *b*, *c*).

The mouth is almost always absent. It is found in the nearly normal G. 1, 8 : iv : '13 (a), and in early stages a stomodæum is present (H. 3, 2 : iv : '13 [a] I. 1, 4 : iv : '13 [a and b]). The pharynx then communicates with the exterior

only by the gill-clefts, which are often well developed (Text-fig. 2, *a*).

The head skeleton is also profoundly modified (except in G. 1, 8 : iv : '13 [a]). Properly speaking, the cranium is entirely absent, with the exception of a small plate of cartilage situated below the débris of the brain, which appears to represent some part of the cranial floor—perhaps the anterior trabecular plate—in G. 1, 8 : iv : '13 (b). The branchial skeleton is, however, better developed in a few cases. In G. 1, 8 : iv : '13 (b) it consists of a wedge-shaped piece embedded in the anterior wall of the pharynx, and a triangular plate placed below the pharynx, with a median depression, in which the thyroid is lodged, a backwardly directed apex, vertical lateral edges, and two pairs of branchial arches. From their relation to the gills and gill-clefts these appear to be the second and third branchial arches. The anterior piece then represents the first branchial and the hyoid, with, perhaps, an admixture of mandibular (quadrate) elements. Bundles of myoblasts connect these two pieces to one another and the rudiment of the skull (Text-fig. 4).

In G. 2, 8 : iv : '13 (a) there is a plate under the throat bearing branchial arches, not symmetrically nor fully developed : on one side is a large third branchial and small fourth, second and first branchials, and a hyoid, the last two being united a little way from the median plate ; on the other side are long second and third, short fourth and first branchials, and a short hyoid. In front the plate is produced into two curved pieces, which may be extensions of the hyoid. Bundles of myoblasts pass from this anterior to the more posterior parts of the apparatus.

In G. 2, 8 : iv : '13 (b) there is a plate bearing three pairs of short branchial arches, the first and second being united on each side. In front, embedded in the anterior wall of the pharynx, is a wedge-shaped piece, resembling that of G. 1, 8 : iv : '13 (b), but here united to the main plate. It appears to be hyoidean.

In G. 2, 8 : iv : '13 (g) there is a similar anterior piece,

with, however, irregular anterior prolongation. Behind, and below the pharynx, is a plate with one pair of irregular arches. Lastly, in G. 2, 8 : iv : '13 (c) the branchial skeleton is reduced to a small nodule in the front wall of the pharynx. Gill-slits are absent in this larva.

Suckers are almost invariably absent. They are found, indeed, only in G. 1, 8 : iv : '13 (a), L. 1, 8 : iv : '13 (b) (Pl. 11, fig. 37), and in comparatively early stages, such as H. 3, 2 : iv : '13 (a and b), I. 1, 4 : iv : '13 (a and b) (Text-fig. 5).

The Changes at the Yolk and Blastopore End.

In G. 2, 8 : iv : '13 (b) (Text-fig. 8), and in all embryos which are as much or more malformed, a central region is observable in the mass of yolk-cells in which cell divisions are absent and the yolk-granules fused together. This would seem to be due to withdrawal of plasma. Another consequence of the operation is the delay in the closure of the blastopore, seen, for instance, in G. 2, 8 : iv : '13 (d, e), H. 3, 2 : iv : '13 (b), I. 1, 3 : iv : '13 (a, b, c), I. 1, 4 : iv : '13 (b, c, d, e), and in most of the very severely affected embryos of the L and M series (Pl. 11, figs. 22, 23, 25, 26, 27, 28, 29, 30). In L. 1, 6 : iv : '13 (e and f), in L. 1, 8 : iv : '13 (e), in M. 1, 6 : iv : '13 (b) and in M. 1, 8 : iv : '13 (b and e) the yolk-plug is, however, withdrawn in spite of the very serious arrest of development (Pl. 11, figs. 34, 35, 39, 42).

Fusion of Paired Structures in the Middle Line; the Hind-brain and Spinal Cord, the Myotomes and Notochord.

In those embryos in which the front part of the nervous system has disintegrated, the brain begins at the level of the auditory vesicles or sometimes a little in front of or behind this point (Text-fig. 2 *b*). Inasmuch as the ganglia of v and vii, or the remains of them, are found with the rest of the débris in the head, we must suppose that part only of the hind-brain has escaped destruction.

This part has the structure of the medulla with a thin roof and a thick floor, but is still not quite normal since the floor is excessively thick, often projecting into the lumen, while a mass of white matter occupies the whole of its ventral side, uninterrupted by any cells. The cells lie above this, the spongioblasts next the lumen, the neuroblasts above the fibres. The lumen is itself further dorsal than it should be. The roof is excessively folded.

In this region are found the auditory and vagus ganglia; they often approach one another in the middle line and may meet (G. 2, 8 : iv : '13 [e and g]) (Text-fig. 6).

The auditory vesicles (Text-fig. 2 *b*) are well developed. In G. 2, 8 : iv : '13 (e) each is constricted into two distinct parts, and in G. 2, 8 : iv : '13 (g) the vesicles of opposite sides are united in front of the hind-brain (Text-fig. 6). They are found, of course, in earlier stages (H. 3, 2 : iv : '13; I. 1, 4 : iv : '13 [d]) (Text-fig. 7). The spinal cord (Pl. 12, fig. 47) (Text-fig. 2 *d*) has the same defects as the medulla—that is to say, the lumen is driven dorsally by an excessive thickening of the floor across which runs a continuous band of fibres; next to this comes a layer of neuroblasts, and then the spongioblasts next the lumen (G. 1, 8 : iv : '13 [b], G. 2, 8 : iv : '13 [a, b, c, e, g], M. 1, 6 : iv : '13 [b]). Posteriorly, however, it may be normal (G. 2, 8 : iv : '13 [b], M. 1, 6 : iv : '13 [b]) (Text-fig. 8), and may be normal throughout (L. 1, 6 : iv : '13 [a]; 8 : iv : '13 [b]). The abnormality is interesting; it is as though the lateral tracts of fibres had come down, forced the neuroblasts of the ventral cornua upwards towards the canal, and then met in a continuous ventral band. In other words, there has been a median fusion of paired structures, and the same phenomenon is seen in the fusion below the cord of the spinal ganglia (G. 1, 8 : iv : '13 [b], G. 2, 8 : iv : '13 [a, partial], [b, in front], [c, e, g]). The ganglia, however, still retain their proper relations with the cord in that the fibres of the dorsal root pass upwards from their cells to enter the cord at the side.

Like the ganglia the myotomes unite below the cord

(Text-figs. 2, *c*, *d*, 10, 11) in a mass of fusiform, horizontally placed myoblasts (G. 1, 8 : iv : '13 [b], G. 2, 8 : iv : '13 [a, not posteriorly], [b, c, e, g], I. 1, 4 : iv : '13 [d], M. 1, 6 : iv : '13 [b, d]). There is little doubt that these latter cells by which the junction is effected are cells which should have, but have not, given rise to the notochord; for first the notochord is absent in these cases or only represented by an occasional vacuolation (as in G. 1, 8 : iv : '13 [b], G. 2, 8 : iv : '13 [e and g]), except where some part of the original material has been saved (as in G. 2, 8 : iv : '13 [b]), and a notochord is seen lying above the median conjunctive mass, or where there has been no fusion (as in the hind-end of G. 2, 8 : iv : '13 [a]). In the second place the beginning of the process is seen in such early arrested stages as L. 1, 6 : iv : '13 (c) (Text-fig. 15), M. 1, 6 : iv : '13 (b, d), where the vertebral plate mesoderm of the two sides is continuous across the middle line in a median mass of cells which are already beginning to elongate.

In early stages (of slightly centrifugalised eggs) there is a normal notochord (H. 3, 2 : iv : '13 [b]), and sometimes in others (L. 1, 6 : iv : '13 [a, b] ; 8 : iv : '13 [b] ; I. 1, 4 : iv : '13 [a, b, c]).

Lastly, in one case, already referred to, the two auditory vesicles are united, while a fusion of the auditory and vagus ganglia may also occur.

I am unable to suggest any explanation of this curious change.

The Organs of the Middle Region of the Body.

With the exceptions already noted, the posterior part of the nervous system, the auditory vesicles, and the muscles of the back are well developed. The same may be generally stated of the pharynx, lungs, alimentary canal, heart and blood-vessels, pronephros, germ-cells and tail.

The pharynx is provided with gill-evaginations, some of which may be open (Text-fig. 2, *a*). In G. 1, 8 : iv : '13 (b) all five pairs are present, and the last three (second, third and fourth branchials) are open. In G. 2. 8 : iv : '13 (a) the first

three branchials are present on one side (the second and third open) and on the other the second, third and fourth branchials (the last two open). In G. 2, 8 : iv : '13 (b) the first, second, third and fourth branchials are present on both sides (the last two open on one side, the last hardly open on the other), and in G. 2, 8 : iv : '13 (g) the first, second and third branchials are present on one side (the second and third open), while on the other are the first and second branchials (the second open). Thus the hyomandibular evagination is found only in the first tadpole; in the remainder branchial clefts in numbers which differ in individuals, and on the two sides of the same individual.

The embryos of Series H and I are too young to show gill-clefts. In L. 1, 8 : iv : '13 (b) there are solid outgrowths, but no perforations.

As already pointed out, there is a correlation between the presence of clefts and the development of a branchial skeleton, as would, of course, be expected.

External gills are found in G. 1, 8 : iv : '13 (b), G. 2, 8 : iv : '13 (a, b and g). They are placed very far forwards (Pl. 11, fig. 20). In G. 1, 8 : iv : '13 (b) there is a small opercular fold.

The trachea and lungs (Text-figs. 2 *b*, 6, 10) are found in the older embryos (G. 1, 8 : iv : '13 [b]. G. 2, 8 : iv : '13 [a, b, c, g, but not e]), whose development is not too much arrested. The pericardium and heart (Text-figs. 2 *b*, 13), with the principal blood-vessels, aortæ and cardinal and vitelline veins, are found in the better developed embryos and are usually well formed (G. 1, 8 : iv : '13 [b], G. 2, 8 : iv : '13 [a, b, e, g], L. 1, 8 : iv : '13 [b]), but in G. 2, 8 : iv : '13 (b) the heart is small, in (g) not twisted, and in (e) and L. 1, 8 : iv : '13 (b) very small and solid. Aortæ and cardinal veins may be present when the heart is absent (G. 2, 8 : iv : '13 [c]).

In G. 2, 8 : iv : '13 (d and e) there are irregular blood-vessels (Text-fig. 14 *b*) and a structure, which is possibly the heart, lying on one side of the pericardial cavity. In the younger but fairly normal embryos (H. 3, 2 : iv : '13 [a, b], I. 1, 4 : iv : '13 [a, b, c], L. 1, 6 : iv : '13 [b, c]), the peri-

cardium is still small, and the heart in either a very early stage or quite undeveloped.

The peritoneal cavity is frequently well formed (Text-figs. 2 *c*, *d*, 6, 8) (as in G. 1, 8 : iv : '13 [b], G. 2, 8 : iv : '13 [a, b, c, e, g]), and in communication with the exterior by the pronephros (Text-figs. 2 *c*, 6, 10, 11). The full number of pronephric tubules and funnels is found in G. 2, 8 : iv : '13 (a, b, e, g), but in G. 1, 8 : iv : '13 (b) there are only two funnels on one side, in G. 2, 8 : iv : '13 (c) two only on both sides. The tubules are bathed, as usually, in the capillaries of the posterior cardinal vein. The glomus is found (except in G. 2, 8 : iv : '13 [a and c]). The ducts open into the cloaca (except in G. 2, 8 : iv : '13 [e]).

In the younger embryos (H, I, L. 1, 6 : iv : '13 [a, b, c], M. 1, 6 : iv : '13 [b, d]), only the pronephric ridge is found (Text-fig. 9). In L. 1, 8 : iv : '13 (b) differentiation of the tubules has not gone very far.

The gut is well differentiated (with stomach, liver and intestine) (Text-fig. 2 *c*) ; in G. 1, 8 : iv : '13 (b), G. 2, 8 : iv : '13 (c, g), less differentiated in G. 2, 8 : iv : '13 (a, b, e), L. 1, 6 : iv : '13 (c). In the H and I series little differentiation, has occurred beyond the formation of the liver diverticulum, and the embryos L. 1, 6 : iv : '13 (a), 8 : iv : '13 (b), M. 1, 6 : iv : '13 (b, d) are in the same early, probably arrested, condition.

A proctodæum, not open to the gut in the early or arrested stages, is found always except where the blastopore persists.

Primordial germ-cells are found at the root of the mesentery in G. 1, 8 : iv : '13 (b), G. 2, 8 : iv : '13 (a, b, c, g) (Text-fig. 2 *d*).

A tail is found, provided with a fin, in G. 1, 8 : iv : '13 (b), G. 2, 8 : iv : '13 (a, b, c) (Pl. 11, figs. 20, 21). In the young embryos it is of course only a short stump.

Œdema.

Many of these tadpoles suffer from œdema or an accumulation of fluid in the connective-tissue inter-cellular spaces,

or in cavities (Text-figs. 6, 10, 11, 12). Such an accumulation is seen in the connective-tissue in G. 2, 8 : iv : '13 (a and c), in the cœlom (G. 2, 8 : iv : '13 [b, c, g], in the posterior cardinal vein round the pronephros (G. 2, 8 : iv : '13 [a, g]), in the lymphatics or blood-vessels round the gut and liver (G. 2, 8 : iv : '13 [c]), and in the pronephric tubules (G. 2, 8 : iv : '13 [e]). In G. 1, 8 : iv : '13 (b) there is a large ventral cavity, in front of, but quite independent of, the pericardium, partially divided by a median septum, which is probably due to the same causes. In several cases where the development has been more seriously interfered with, the segmentation cavity persists. This, perhaps, belongs to the same category. The persistent blastocœl may contain a few scattered mesodermal cells, but otherwise retain its original character, its front wall being formed of the small ectodermal cells of the animal hemisphere, its hind wall of the large yolk-cells (M. 1, 8 : iv : '13 [c]), or the mesodermal cells which have advanced into it may give it a lining of its own, incomplete (M. 1, 8 : iv : '13 [b], L. 1, 8 : iv : '13 [d] [1]), or complete (M. 1, 8 : iv : '13 [a], G. 2, 8 : iv : '13 [d]) (Text-fig. 14 *a*). The cavity in question has no communication with the alimentary canal, which is, indeed, in most of these cases very small, or restricted to the blastoporic groove.

The Changes after Severer Treatment.

While it is thus possible, if the centrifugal force applied be not too great, to obtain tadpoles which, though deformed anteriorly and in the region of the blastopore, are yet more or less normal in the middle portion of the body and in the tail, in individuals which have suffered more seriously there is witnessed a gradual loss of structure, until eventually no more differentiation occurs than is involved in the production of some dorsal and ventral mesoderm and a slight blastoporic overgrowth.

The heart usually goes before the pericardium, or, to express it in a better way, the latter may be developed while the former

is not. Thus in L. 1, 8 : iv : '13 (b) the heart is solid, the pericardium large, and in I. 1, 4 : iv : '13 (c), L. 1, 6 : iv : '13 (b, c), the former is absent while the latter is present. In the more seriously injured embryos neither is found, though blood-vessels may be (G. 2, 8 : iv : '13 [d]). The pronephros holds out perhaps a little longer, as it is formed not only in the embryos just mentioned, but also in M. 1, 6 : iv : '13 (b, d), which possess no pericardial cavity. The peritoneal cavity appears to persist in these same embryos, but is not found in the more degenerate individuals (except possibly in L. 1, 8 : iv : '13 [e]). The absence of the heart and pericardium in L. 1, 6 : iv : '13 (a), while the nervous system and auditory vesicles are present, may indicate that in general the former organs are affected before the latter. The auditory vesicles are found almost as long as the central nervous system persists. Indeed, in G. 2, 8 : iv : '13 (e) they are well developed, while the hind-brain and spinal cord are reduced. On the other hand, in I. 1, 4 : iv : '13 (e) and in L. 1, 6 : iv : '13 (c) they have disappeared, while the hind-brain and spinal cord have remained.

The successive steps in the degeneration of what is left of the nervous system are easy to follow. The hind-brain has a very small lumen in I. 1, 4 : iv : '13 (d), is nearly solid in I. 1, 4 : iv : '13 (a, b, c, e), L. 1, 6 : iv : '13 (a), M. 1, 6 : iv : '13 (b, d), and quite solid in G. 2, 8 : iv : '13 (e), L. 1, 8 : iv : '13 (c) (2). The spinal cord is solid in the last-mentioned and nearly so in the others ; in L. 1, 6 : iv : '13 (c) it is still open behind (Text-figs. 15, 16). In all other cases there is no sign of the nervous system at all (Text-fig. 17). The gut may remain—even if only as an archenteron—when the other organs have disappeared. Thus it is found as a narrow but fairly long cavity in L. 1, 6 : iv : '13 (c), M. 1, 6 : iv : '13 (b, d), which still possess a nervous system (Text-fig. 16). In G. 2, 8 : iv : '13 (f), L. 1, 6 : iv : '13 (f) (Pl. 11, fig. 35), L. 1, 8 : iv : '13 (d, e) (Pl. 11, fig. 39), M. 1, 6 : iv : '13 (a, c), and M. 1, 8 : iv : '13 (a, b, c, d, e) (Pl. 11, figs. 42, 43)—none of which have any nervous system—it is a very short cavity, opening by the proctodæum or by the blastopore. In G. 2, 8 : iv : '13 (d) (Pl. 11, fig. 22), L. 1, 6 : iv :

'13 (d), L. 1, 8 : iv : '13 (a, b) (Pl. 11, figs. 36, 37), (c, f, g) (Pl. 11, figs. 40, 41), it is reduced to the blastoporic involution (Text-fig. 14 *b*), while in L. 1, 6 : iv : '13 (e) it is altogether absent, though a proctodæum is present.

The tail remains in many of these extremely stunted forms as a longer or shorter stump, as in G. 2, 8 : iv : '13 (d), L. 1, 6 : iv : '13 (f), L. 1, 8 : iv : '13 (a, b, e), M. 1, 6 : iv : '13 (a, b, d), M. 1, 8 : iv : '13 (a, b, c, e), and it is sometimes double (L. 1, 6 : iv : '13 [e], L. 1, 8 : iv : '13 [f, g]). The double rudiment of the tail is seen of course in those cases where it is represented only by two caudal swellings (I. 1, 4 : iv : '13 [c], L. 1, 6 : iv : '13 [a], L. 1, 8 : iv : '13 [c]) (figs. 29, 31, 38). These tails, though devoid of nervous system and notochord, may yet display traces of a metameric segmentation of the mesoderm (as in L. 1, 8 : iv : '13 [f, g], M. 1, 8 : iv : '13 [b]) (Text-fig. 18).

In the rest there is not even a tail. Dorsal and ventral mesoderm are, however, always developed. The dorsal mesoderm may give indications of the differentiation of a median notochordal tract (L. 1, 6 : iv : '13 [g], 8 : iv : '13 [c], [1]).

(B) On the Chemical Nature of the Substances in the Frog's Egg which may be Separated from one another by the Centrifuge.

The inquiry into the chemical nature of the substances in the several layers which appear in the centrifuged egg can only be prosecuted with any hope of success by centrifuging a large quantity of egg-material *in vitro*. For this purpose the eggs must be obtained free from their coating of mucin-jelly, that is, before they have entered the oviduct.

Such eggs are not, strictly speaking, in quite the same physiological condition as the fertilised eggs, inasmuch as in them the maturation divisions have not yet occurred. If, however, the eggs be taken after their release from the ovary—while they are still in the peritoneal cavity—or immediately before that release, it will be found that the germinal vesicle has already broken down and dispersed its

contents into the cytoplasm, while the first polar spindle has come to the surface of the egg. In their cytoplasm such eggs are probably not so very different from those that have become completely mature.

My first idea was to employ only cœlomic ova, but I soon found that I could not obtain anything like a sufficient quantity of material in this way, since at any one moment but few eggs are found in the body-cavity, some being still retained in the ovary, while others are already in the oviduct. I, therefore, adopted the plan of removing the ovaries, washing them well in Ringer's solution to remove blood and peritoneal fluid, and then leaving them in a quantity of the same solution at a low temperature until the eggs dropped out. I was able to get considerable numbers of eggs by this method, which seems to me to be preferable to that employed by McClendon; this, as already pointed out, involves the inclusion of all the young ova with the old ones.

From the eggs so obtained the Ringer's solution was poured away as completely as possible. The ova were then ground to a fine pulp in a mortar, and this egg-pulp was centrifuged. In the first experiments a fairly high velocity was used (about 3200 revolutions a minute) for about twenty minutes; in later experiments, to which I shall refer presently, different and lower accelerations and exposures were employed and their effects compared.

The centrifuged mass becomes separated into three distinct layers:

1. A centripetal dark yellowish-grey layer.
2. A middle light grey or opalescent layer.
3. A very thick black centrifugal layer.

The first of these consists of fatty substances and some protein, the second of proteins and glycogen, and the third of pigment and yolk with an admixture of fatty substances.

Layer 1.

(i) This layer is slimy and viscid, hardened at its surface into a thin crust.

(ii) Microscopical examination: Pigment-granules, refringent

globules entangled into angular masses in some other material, and some fluid.

The refringent globules are soluble in chloroform and acetone, and blacken with osmic acid. They appear, therefore, to consist of a fatty material. The material in which the globules are entangled and by which they are partly obscured is soluble in 1 per cent. NaOH. The globules then become more evident.

Some of the globules are nearly or quite colourless, others of a yellow colour. There are no yolk-granules in this layer.

(iii) When this stuff is boiled for some time in alcoholic potash a solution of soap is formed which may be salted out with NaCl. By $CaCl_2$ the soap is precipitated, and on the addition of acetic a layer of fatty acid rises to the surface.

(iv) A portion of the material was mixed with ·75 per cent. NaCl and filtered.

A. The filtrate—

(1) Filters quickly.
(2) Is opalescent.
(3) Gives Millon's reaction slightly.
(4) Gives Heller's reaction slightly.
(5) Gives the xanthoproteic reaction slightly.
(6) Gives a slight heat coagulum.
(7) Gives a slight glycogen reaction with iodine.

B. The residue—

(1) Gives Millon's reaction.
(2) Gives the xanthoproteic reaction.
(3) Dried to a black colour and washed well with ether. The ether becomes yellow. The residue is now grey.
(α) This grey residue gives the xanthoproteic reaction.
(β) This residue was now washed with hot alcohol, dried and incinerated. The ashes were dissolved in dilute HNO_3. On the addition of ammonium molybdate yellow crystals of ammonium phosphomolybdate appear.

(v) Another portion of this material was placed in alcohol.

a. A flocculent coagulum appears at once. The coagulum gives Millon's reaction.

b. The material was then boiled repeatedly in alcohol. The alcohol becomes yellow, and several large yellow globules fall to the bottom without dissolving.

Filtered hot—

A. (i) The filtrate, which becomes cloudy on cooling, was now boiled

for half an hour with BaOH, the baryta soap filtered off, the barium removed by passing CO_2, the $BaCO_3$ filtered off and the filtrate evaporated to dryness.

A minute fragment of the evaporate placed on a slide under a cover-glass in IKI showed clouds of black globules and then rectangular black crystals. This is choline ennea-iodide, and proves that part at least of the fatty material is lecithin.

(ii) On evaporating a portion of the alcoholic filtrate a yellow-brown residue is left. This residue is soluble in ether, but not in acetone. A small piece placed in water slowly swells and puts out finger-shaped processes.

When incinerated and dissolved in dilute HNO_3 it gives crystals of ammonio-phosphomolybdate with ammonium-molybdate and coffin-lid crystals of ammonio-magnesio-phosphate with magnesia mixture.

B. The residue washed with ether. The ether becomes yellow.

(i) While acetone gives no precipitate with this yellow filtrate, the choline reaction can nevertheless be obtained from the dried residue.

(ii) The second residue, after washing with ether, was dried and placed in 1 per cent. NaOH, in which it dissolves. The solution—

(α) Gives the xanthoproteic reaction.

(β) Gives Millon's reaction.

(γ) Gives a pink biuret reaction.

(δ) Does not give the iodine reaction for glycogen.

The centripetal layer therefore contains fatty substance, protein and a little glycogen. Part of the fatty substance is lecithin, which can be precipitated by acetone from alcoholic but not from ethereal solution, which will give choline, and from which phosphorus may be obtained. Part seems to be fat, as some of the globules are soluble in acetone.

The proteins seem to include a globulin, but others are possibly present; for instance, the solid protein in which the fat-globules are embedded, which may be the same as the eosinophilous coagulum seen in the vacuoles of the egg-cytoplasm. The phosphorus obtained from the protein-containing residues may be due to inorganic phosphates or perhaps to a phospho-protein or a nucleo-proteid. No purine

bases have, however, so far been satisfactorily demonstrated in any constituent of this layer.

This layer obviously corresponds to the grey patch in the highly centrifugalised eggs.

The admixture of a good deal of melanin pigment is an additional difficulty in the investigation of the proteins of this layer.

Layer 2.

A. (i) This layer is opalescent and liquid.

(ii) The greyish colour is not due to pigment, but to angular masses enclosing refringent globules of fatty material. These masses are not very numerous. They are soluble in 1 per cent. NaOH.

(iii) The liquid plasma is coagulated by alcohol, the coagulum being finely granular.

(iv) Distilled water produces a finely granular precipitate, the granules being arranged in a reticulum in which the angular masses are emmeshed. The greater part of the plasma remains liquid.

(v) The plasma may be coagulated by heat.

(vi) It may be precipitated by sublimate, and by 2 per cent. acetic.

(vii) HNO_3 gives a white precipitate. This becomes yellow on heating, and with NH_3 an intense yellow.

B. A portion of this layer is dissolved in distilled water and filtered.

(i) The filtrate—

(*a*) Is perfectly clear under the microscope.

(*b*) Is slightly alkaline.

(*c*) Is heat coagulable.

(*d*) HNO_3 produces a slight opalescence.

(*e*) Gives the xanthoproteic reaction.

(*f*) Gives Millon's reaction.

(*g*) Gives a purple biuret reaction.

(*h*) Gives a slight glyoxylic reaction (Adamkiewicz).

(*i*) Boiled with 40 per cent. NaOH and treated with lead acetate gives no sulphur reaction.

(ii) The residue, incinerated, gives the ammonium molybdate phosphorus reaction.

C. A portion of this layer is dissolved in ·75 per cent. NaCl and filtered; it filters slowly.

(i) The filtrate—
  (*a*) Is opalescent.
  (*b*) Is alkaline.
  (*c*) Gives a heat coagulum.
  (*d*) Gives no precipitate with 2 per cent. acetic.
  (*e*) Gives Heller's HNO_3 reaction.
  (*f*) Gives the xanthoproteic reaction.
  (*g*) Gives Millon's reaction.
  (*h*) When boiled with H_2SO_4 the vapours (of furfural) turn anilin acetate red.
  (*i*) Acidified with acetic, iodine gives an abundant red colour, which disappears on heating but reappears on cooling.
  (*j*) This glycogen reaction is not given after the liquid has been digested with saliva.
  (*k*) After incineration gives the ammonio-phosphomolybdate and ammonio-magnesio-phosphate reactions.

(ii) The residue, dried and washed with ether—
  (*a*) Gives the xanthoproteic and—
  (*b*) Millon's reactions.
  (*c*) Washed again, in hot alcohol, the residue, incinerated gives the two phosphorus reactions.

It appears, therefore, that the second layer contains proteins, a good deal of glycogen, presumably in solution, and a small quantity of fatty substance, of which a part may be lecithin.

The evidence, so far as it goes, seems to point to the existence of at least two heat coagulable proteins, of which one is soluble in water, the other not. There is also the solid protein, in which the fat-globules are entangled. The phosphorus may be due to inorganic phosphates, or to phosphoproteins or to nucleo-proteins, but no satisfactory proof of the existence of the last has been obtained.

This layer is represented in the eggs by the white circle outside the grey patch.

Layer 3

A. (i) Is thick and pasty.
  (ii) Microscopically examined, yolk-granules, pigment and fat-globules are seen.

The yolk-granules are soluble in—

(*a*) NaCl 10 per cent., 5 per cent., $2\frac{1}{2}$ per cent., but in $1\frac{1}{4}$ per cent. only swell a little and lose their refringency without disappearing. In saturated solution the yolk-granules swell and become less refringent, but remain distinct.

(*b*) $(NH_4)_2SO_4$ $\frac{2}{5}$ saturated solution, but not in $\frac{1}{2}$ saturated or saturated solution.

(*c*) NaOH 1 per cent.

(*d*) Na_2CO_3 1 per cent., 2 per cent.

B. A watery extract is made and filtered.

The filtrate—

(*a*) Is opalescent.

(*b*) Is alkaline.

(*c*) Is coagulable by alcohol.

(*d*) When acidified and boiled gives a heat coagulum.

(*e*) Gives Heller's reaction.

(*f*) Gives the xanthoproteic reaction.

(*g*) Gives Millon's reaction.

(*h*) Gives a purple biuret reaction.

(*i*) Re-filtered, the filtrate still gives a heat coagulum, and Heller's, Millon's, and the xanthoproteic reactions.

(*k*) Gives no glycogen reaction with iodine.

C. An extract is made in ·75 per cent. NaCl and filtered.

The filtrate—

(*a*) Is opalescent.

(*b*) Is alkaline.

(*c*) Gives a heat coagulum when acidified and boiled.

(*d*) With HNO_3 gives a cloudy precipitate, which partially clears on boiling and reappears on cooling.

(*e*) This turns yellow on addition of NH_3.

(*f*) Gives Millon's reaction.

(*g*) Gives a purple biuret reaction.

(*h*) Is precipitated by distilled water.

(*i*) Is precipitated by alcohol.

(*j*) Does not give the anilin acetate reaction.

D. An extract is made with 1 per cent. NaOH and filtered.

(i) The filtrate gives a precipitate with 2 per cent. acetic, completely when the liquid has been neutralised.

(ii) Re-filtered, the filtrate—

(*a*) Is coagulated by alcohol.

(*b*) Gives the xanthoproteic reaction.

(*c*) Gives Millon's reaction.

(*d*) Gives a purple biuret reaction.

E. A quantity of the material is ground up in a mortar and washed with ether. The ether becomes yellow.

It is then boiled in alcohol, which extracts still more fatty substance.

The residue—

(*a*) Dissolved in 10 per cent. trichloracetic acid, and filtered. The filtrate gives no phosphorus reaction.

(*b*) Incinerated and the ashes dissolved in dilute HNO_3;

(1) Ammonium-molybdate gives crystals of ammonio-phosphomolybdate.

(2) The crystals washed in water and dissolved in NH_3. Magnesia mixture gives crystals of triple phosphate.

(*c*) Boiled with H_2SO_4. The fumes do not give the anilin acetate reaction.

F. A quantity of the stuff is treated with ether and hot alcohol until the fat and lecithin has been extracted.

It is then subjected to the following treatment, borrowed with slight modification from Fridericia, in order to see whether purine bases are present:

[(1) Hydrolysed by boiling for fifteen hours with 1 per cent. H_2SO_4.

(2) NH_3 added till alkaline.

(3) Boiled till no alkaline vapours are given off.

(4) Two per cent. acetic is added till the reaction is acid, and the liquid is boiled.

(5) The coagulated proteins are filtered off.

(6) Equal parts of 40 per cent. sodium bisulphite and 10 per cent. copper sulphate are added. The whole is boiled for three minutes.

(7) Filtered. The residue washed repeatedly with boiling water until the water is no longer blue.

(8) Filter-paper and residue are now put back into the same flask that was used for the copper precipitation, water is added, the whole brought to the boiling-point, and excess of sodium sulphide added.

(9) Acidified with acetic and the H_2S boiled off; filtered. The residue washed with boiling water which is added to the filtrate.

In this filtrate are the purine bases if any.]

The copper precipitate obtained from the material of layer 3 was a bright Indian red colour. The final filtrate (9) gave the following reactions:

(*a*) NH_3AgNO_3 added. A gelatinous precipitate came down at once. This is the silver compound of the purine base.

(*b*) The precipitate of (*a*) was washed and dissolved in hot 33 per cent. HNO_3. A crystalline precipitate slowly settled.

(*c*) Evaporated with HNO_3 to dryness it became yellow. The addition of NaOH turned this a bright red, which colour heat converted to a brownish-red. This points to xanthine.

(*d*) Chlorine water and a drop of HNO_3 was added. The whole is evaporated. The residue was white. The addition of NH_3 turned this first yellow then dark red on heating. This again points to xanthine.

(*e*) It was precipitated by ammoniacal basic lead acetate but not by basic lead acetate alone.

(*f*) It was precipitated by NaOH; the precipitate was soluble in excess.

(*g*) With HCl a white crystalline precipitate was given.

(*h*) With Zn and HCl no red colour was given.

While, therefore, there is no doubt that a purine base may be extracted from the yolk-granules, it is not certain which one is present. The reactions appear to point to xanthine.

The third layer consists, therefore, of pigment and yolk, and some fat. There is no glycogen. There may be a little plasma left between the yolk-granules, which would give the proteins found in the watery extract.

The solubilities of the yolk-granules point to their protein being a globulin or a nucleo-protein. They certainly consist of or contain a nucleo-protein, from which a purine base, probably xanthine, can be obtained.

The third layer obviously corresponds to the pigmented yolk region of the centrifuged egg.

These data do not, of course, pretend to be in any sense a complete account of the chemistry of the frog's ovum, but they are, I hope, a beginning which may serve as the basis of future work. Even as they stand they may throw some light on the relation between the stratification of the egg-cytoplasm and the abnormal development of the embryo.

Before concluding this section I will refer briefly to the results of one or two other experiments in which the thickness of the layers was determined in egg-pulp centrifuged at different

speeds, and at the same time, and for the same length of time, as the eggs of the series H. 1, 2 and 3 and I. 1 and 2.

These results are embodied in the accompanying table :

	Thickness in millimetres :		
	Layer 1.	Layer 2.	Layer 3.
H. 1 (10 minutes at speed III)	8		24
H. 2 (10 minutes at speed II)	4		26
H. 3 (ten minutes at speed I)	2		22
I. 1 (10 minutes at speed IV)	1	$2\frac{1}{2}$	$27\frac{1}{2}$
I. 2 (30 minutes at speed IV)	1	5	31

It was not possible in the H series to distinguish accurately the boundary between the first and second layers.

In the next table the relative volumes of layers 1 and 2 taken together and of layer 3 are given, and the ratios of the volume of the third to that of the others.[1]

	Relative volumes.		Ratio $\frac{\text{layer 3.}}{\text{layers 1 and 2.}}$
	Layers 1 and 2.	Layer 3.	
H. 1	8	$21\frac{1}{2}$	2·7
H. 2	4	$23\frac{1}{2}$	5·9
H. 3	2	$19\frac{1}{2}$	9.75
I. 1	$3\frac{1}{2}$	25	7·14
I. 2	6	$28\frac{1}{2}$	4·75

The relative volume is a measure of the disturbance. Since the embryos of H 3 develop nearly normally, while those of I. 1 develop, but not so normally, but the remainder not at all, it may be said that centrifuged eggs are only able to develop when in a mass of egg-pulp centrifuged at the same acceleration and for the same time the combined volumes of the

[1] In calculating the volume allowance must, of course, be made for the hemispherical bottom of the tube.

layers of fat and plasma are not more than one seventh of the volume of the layer of yolk.

I have only to add that the few quantitative determinations I was able to make show, as we should expect, that the water content and the fat content of the third layer diminish with increased centrifuging. The fat was extracted in a Soxhlet apparatus. I give the figures in tabular form :

	Percentage of dry substance in layer 3.	Fat in layer 3 given as a percentage of the dry substance.
H. 1	32·6	22·4
H. 2	31	24·4
H. 3	29·9	—
I. 1	32·4	—
I. 2	34·9	—
Egg-pulp not centrifuged	30·4	25

That there is a smaller percentage of dry substance in the third layer of H. 3 than in the uncentrifuged egg-pulp must be attributed simply to the fact that different lots of eggs were used in these two determinations :

(c) Conclusions to be Drawn from the Foregoing Experiments.

The egg of the frog has a structure which depends on a certain arrangement of visible materials—the plasma, the glycogen, the yolk and the pigment—this arrangement being such that the plasma and the glycogen gradually decrease in concentration from one point on the surface towards the diametrically opposite point, while the yolk gradually increases in concentration in the same direction. The pigment lies in a superficial sheet in the plasmatic two thirds of the egg. In respect of the distribution of these substances, therefore, the egg has a polar structure, or polarity, or is radially symmetrical about an axis with unlike poles. As a result of fertilisation this polar is replaced by a bilateral structure

when the grey crescent appears on one side of the egg at the margin of the pigmented area.

To this original polar and superimposed bilateral structure the structure and symmetry of the embryo are definitely related in ordinary development in such a way that the head of the embryo is formed near that pole towards which the concentration of the plasma is increasing—the animal pole—the blastopore closes near the vegetative pole, that towards which the concentration of the yolk is increasing, while the dorsal side is that on which the grey crescent appeared. Ventral, right and left sides are therefore also simultaneously determined.

From Pflüger's experiment, and the variant of it in which a centrifugal force is substituted for gravity, it appears that a new polarity, and, indeed, a bilateral symmetry, may be conferred upon the egg merely by redistributing the heavier and lighter constituents of the cytoplasm. The new polar structure resembles the old in being determined by a similar graduated arrangement of materials, and to it the segmentation of the egg and the symmetry of the embryo bear precisely the same relation as do the cleavage furrows and the parts of the embryo to the structure of the egg in normal development.

We might reasonably suppose, therefore, that any disarrangement of these materials, severe enough to alter their normal graduation, would bring about a distortion of development or might even prevent it altogether. And this, as we have seen, actually occurs.

By the centrifuge the materials of the egg are driven past one another in opposite directions, the fat towards the centripetal (animal) pole, with some entangled pigment and some plasma, the yolk and pigment towards the centrifugal (vegetative) pole, while the movement of the plasma is opposite at one end to that of the fat, at the other to that of the yolk.

Where the separation of materials is nearly or quite complete no development is possible nor even a normal cleavage. In

less severely centrifuged eggs, while there must in any case be an excess of fatty substances round the animal pole, and a deficiency of plasma, glycogen and yolk, an excess of yolk and a deficiency of plasma and fat round the vegetative pole, it is possible that in the equatorial region the relative concentration of the different stuffs, their graduation, may remain normal. Such eggs may develop, but monstrously.

The irregularities of cleavage and development may be directly traced to this disturbance.

As far as cleavage goes it appears that yolk deprived of its plasma cannot divide, as, of course, is seen in normally meroblastic eggs, though nuclei may be present. In the enlargement of these yolk-nuclei, their excess of chromatin and their apparently amœboid movements, there is an interesting analogy with the yolk-nuclei of Elasmobranch and Teleostean ova. The distortions of development are seen primarily at the head and blastopore ends—that is, in the parts developed from materials situated at the animal (centripetal) and vegetative (centrifugal) poles. In front there is a fatty vacuolation of the ectoderm and of other organs, which increases in the more severely handled eggs. This is obviously due to the accumulation of fat around the animal pole. In the yolk towards the other end there is seen a mass where there are no cell-divisions and the yolk-granules have fused. This is to be attributed to the deficiency of plasma. Where the derangement of materials has been greater the front part of the head is degenerate, and the blastopore fails to close, the yolk-plug remaining exposed. The first of these malformations must be assigned to the excess of fat and lecithin, the deficiency of plasma and possibly of glycogen, and probably also of yolk, since the yolk contains a store of nucleo-protein which is presumably normally used in the production of fresh nuclear material. The persistence of the yolk-plug, on the other hand, is caused ultimately by a deficiency of plasma. In the normal process of the closure of the blastopore the yolk-cells are not merely passively pushed beneath the overgrowing blastoporic lip, but creep actively inwards, as Kopsch

showed long since. Anything which diminishes their vitality, as, for example, in experiments of another kind, heat or solutions of salt and other substances, will inhibit the process and the yolk-plug will persist. In the present case it is the deficiency of the plasma; the cells are too large and overloaded with yolk. But while at the animal and vegetative poles, or head and blastopore ends, the development is being distorted in this way, the middle region of the body may remain fairly normal. As already pointed out, the distribution of materials may remain approximately normal in the equatorial tract while altered at the poles, and the development of the middle part be regular for this reason. With more centrifuging, however, the usual distribution of stuffs in this region also is lost and then the power of development disappears; the heart, pronephros, auditory vesicles, nervous system and alimentary canal all go, and we are left with an embryo in which no development has occurred beyond the differentiation of some mesoderm. The significance of the experimental results obtained by Konopacka is now apparent. When the egg is centrifuged in an early stage (prior to fertilisation), sufficient time (several hours) elapses before cleavage begins, a return of the materials to their original position is possible, and development is normal. But when the disturbance takes place immediately before cleavage, or during its early phases, there is no time for recuperation and there are many abnormalities. That such a return does take place, or at least begins to do so, is shown by the blurring of the zones and rings, even in highly centrifuged eggs, by the time cleavage begins.

A certain arrangement of most of the visible materials of the cytoplasm appears therefore to be a condition of normal development: the concentration of the plasma, glycogen and yolk must be graduated in a certain way, that of the fat must be uniform. The position of the pigment is alone inessential, as Morgan has pointed out. But with this exception the factors to which the egg owes its visible polar structure are also the causes of the production from that egg of a normal organism.

[Though it does not fall within the scope of these experiments, it seems probable that the relation between the bilateral structure of the ovum and the bilateral symmetry of the embryo might be expressed in similar terms. It seems likely that at the time of the formation of the grey crescent there is some redistribution of the materials in the cytoplasm : it is, indeed, known that the grey crescent is due to an immigration of pigment.]

The visible materials of the egg are partly living, the plasma, partly not, the yolk, glycogen and fat. Though the latter are not properly to be termed organo-genetic, yet they condition development in the manner described. In the living plasma, on the other hand, there may be distinct organo-genetic bodies, arranged in a certain way, and each causally related to the formation of some particular organ, and the disarrangement of these would necessarily involve malformation. No evidence for their existence is, however, brought forward by these experiments, and we must postpone the discussion of this problem until we have inquired how far in other cases also a certain arrangement of materials not only bestows a polarity upon the egg, but is also a condition of the normal development of the embryo.

(III) ON THE RELATION BETWEEN THE CYTOPLASMIC STRUCTURE OF THE EGG AND THE DEVELOPMENT OF THE EMBRYO IN GENERAL.

The effect upon development of deranging the cytoplasmic materials of the egg has now been investigated in the ova of several Invertebrates.

We turn first to the experiments carried out by Lyon, and by Morgon and Spooner, upon the eggs of the sea-urchin, Arbacia, in which there is a diffuse red pigment.

If the ripe but unfertilised ovum be strongly centrifuged (f = 6400 g) four strata appear. The red pigment passes to the centrifugal pole ; next to this is a grey granular layer, blackened by osmic acid, then a fluid hyaline layer in which

lies the nucleus, while the centripetal pole is occupied by a cap of opaque white material. The new axis of stratification which is thus produced by the operation may make any angle with the original axis as determined by the micropyle. When removed from the centrifuge the strata begin to re-mingle, but the first and fourth return to their original positions very slowly, if at all. The second and third layers, on the other hand, intermingle with one another rapidly, and it is apparently necessary that they should do so before segmentation and development can occur, for if the egg be broken into two portions between them, then neither portion can be fertilised (Lyon).

In segmentation it is the axis of stratification which determines the direction of the furrows, since the first three, which are at right angles to one another as in the normal egg, either include or are at right angles to this axis, or to put it in another way, the axis of stratification coincides with one line of intersection between some two of these three divisions. At the next division the micromeres are formed at that intersection of two furrows which is at the anti-micropylar pole or nearest to it (when the axis of stratification is oblique to the original egg-axis). It appears therefore that some invisible polarity of the egg has remained unaffected by the centrifugal force, and that this determines, or at least helps to determine, the symmetry of the embryo, since the micromere pole becomes the blastopore pole and the original egg-axis the gastrula axis, or as nearly as possible (Morgan). The pigment is found in any part of the larva, right or left, dorsal or ventral, anterior or posterior. It is not, therefore, essential to the development of any particular part.

It may be added here that the yellow pigment band of Strongylocentrotus is equally unnecessary. Normally it is subequatorial and passes into the archenteron, but it may be meridional or oblique to the egg-axis and so become incorporated wholly or partly in the ectoderm (Garbowski).

Experiments of a like kind on other eggs have yielded very similar results, for while the existence of an invisible structure

has been revealed, a structure which is not disturbed by the centrifuge and is definitely related to the subsequent differentiation, that differentiation has been shown to be independent of some at least of the visible constituents of the cytoplasm.

Thus Lillie, by centrifuging the egg of Chætopterus during the first maturation division, produced in it three layers—a small grey cap at the centripetal pole, a clear layer, and a yellow granular hemisphere (on the centrifugal side). These strata, it was found, might occupy any position with regard to the egg-axis (as defined by the polar bodies), yet in fertilisation the sperm always entered at the vegetative pole and cleavage was always normally related to that axis. The grey cap is derived from the contents of the germinal vesicle, the clear band from the microsomes of the endoplasm, and the yellow granules from the coarser endoplasmic constituents. It would be interesting to know something of the further fate of these centrifuged eggs.

This we do know in some other cases.

The ovum of the Lamellibranch Cumingia contains a red pigment and an oily green material, both scattered through the cytoplasm. When the egg is centrifuged during the first polar division (Morgan) these go to opposite poles, the red pigment to the centrifugal, the green oil to the centripetal. Between the two is a broad hyaline layer. Maturation proceeds and the polar bodies are extruded. With the egg-axis, as so determined, the axis of stratification may make any angle. Fertilisation occurs, and in the subsequent cleavage the planes of division bear the normal relation to the axis of the egg. The strata persist so that the red pigment may be in the AB or the CD cell and so on, the green oil on the opposite side. Development follows, and these two coloured materials are found in the trochophore larva in any position opposite to one another. The structure of the trochophore has the normal relation to the cleavage system and therefore to the original axis. There does, however, appear to be some tendency for the green stuff to redistribute itself.

So, again, in Pulmonate eggs (Physa, Planorbis, Limnæa) Conklin has by the same means produced three strata—a grey finely granular zone at the centripetal pole, a narrow clear band, and a yellow granular centrifugal hemisphere. When segmentation and development take place the strata make any angle with the first and subsequent furrows, and any angle with the principal planes of the embryo. Conklin has, however, added the important observation that the possibility of obtaining a normal development is largely dependent on the redistribution to or towards their original positions of some of the disturbed cytoplasmic materials, for it is only when the operation is performed prior to maturation or during its earliest stages—only, that is, when some time elapses between the operation and cleavage—that development is afterwards normal. Eggs centrifuged during the extrusion of the first polar body or later either die or give rise to monstrous embryos. It appears, further, that during the interval the clear substance disappears into the grey or the yellow layer or both—a readjustment which cannot occur unless sufficient time be allowed.

In another mollusc, Crepidula, on the other hand, as well as in the Ascidian Cynthia, Conklin has found it possible, by prolonged centrifuging, to shift the original polar axis (the position of which in the experiments just quoted is left unaltered) without prejudice to normal development. The symmetry of cleavage and of differentiation are, it seems, determined by the new polarity, as in the frog's egg.

In Cyclops (Spooner) the centrifuge separates the cytoplasm into three similar zones—a greenish-white layer at the centripetal pole, a middle clear stratum, and the blue yolk-granules. These eggs are stated to develop normally, even when continuously centrifuged.

From Hegner's observations it appears that in centrifuged beetle eggs the embryo is developed at the centripetal end, whether this be the morphologically anterior or posterior end; also that when the eggs are centrifuged violently enough to produce well-marked layers the development is abnormal.

In the Rotifer Hydatina (Whitney) the polar zones are pink and grey, the middle clear as in the foregoing instances. The stratification may have any relation to the original axis, and the first cleavage is, as in the normal egg, transverse to this axis. Normal young are produced, become mature and reproduce in their turn.

Lastly, in the centrifuged egg of Ascaris similar strata appear. The normal egg is telolecithal. There are in the cytoplasm also some clear spherules and pigment-granules. The layers that appear after centrifuging are a sheet of yolk at the centripetal end (the yolk is here lighter than the cytoplasm), a layer of clear spherules, clear plasma, and finally at the centrifugal pole a cap of brown pigment. When strongly centrifuged the egg becomes flattened against the slide on which it is placed (the centrifugal force is perpendicular to the slide), and, if still subjected to the action of the force, the fertilisation spindle places itself at right angles to the direction of the latter—that is, parallel to the stratification (and in the clear zone), and the division is meridional. If, on the other hand, the egg is removed from the machine (or is less strongly centrifuged), it resumes or keeps its spherical shape, and the spindle returns more or less completely to its proper axial position, and the first division is equatorial (or oblique).

It is suggested by Boveri and Miss Hogue (to whom the experiment is due) that there is an invisible polarised structure in the cytoplasm which is not affected by the operation and with the axis of which the stratification of the movable substances can make any angle. Into the axis of this invisible polarity the spindle is supposed to return if and when the egg is allowed to resume its spherical shape. The facts do not appear to necessitate this view, for when placed on the machine the whole egg rotates inside its shell until the heavier animal pole is centrifugal, and then the stratification of the cytoplasmic materials begins. As long as the force is operating the spindle is compelled to place itself parallel to the stratification, but when released from the force,

returns or attempts to return to its usual position, namely, in the egg-axis, that is, in the stratification axis. The obliquity of the spindle in those cases where the return to the normal position is incomplete would then be the result of two tendencies, at right angles to one another, urging the spindle to place itself the one perpendicular, the other parallel to the stratification.

When the spindle returns (more or less completely) to its normal situation the division is equatorial or oblique, and a normal embryo is developed, in spite of the stratification. When, however, the spindle remains parallel to the stratification the first division is meridional, and each cell behaves as the P_1 (vegetative) cell of an entire ovum. (The greater part of the pigment cap is usually extruded from these eggs at the centrifugal pole as a "ball.") Each half blastomere divides into two, which can be recognised as *E. M. St.* (endo-meso-stomoblast) and P_2 (meso-gonoblast), by the chromosomes being diminished in the one, intact in the other, and by their subsequent behaviour, and so gives rise to what is essentially a blastula without ectoderm. (The ectoderm is normally derived from the absent S_1, the sister cell of P_1.) It might be imagined that the ectodermal material had been extruded with the "ball," but apparently this is not so, since development is the same when (as may happen) no ball is extruded.

Now, when we survey the results of these experiments, it certainly does seem at first sight as though the most incontrovertible proof had been brought forward for the existence of a polar structure in the cytoplasm, invisible, not to be shifted by the centrifuge, and not therefore dependent on any graduation or stratification of heavier and lighter materials in opposite directions, a structure, moreover, to which the symmetry of the embryo is definitely related, while it bears no such relation to the visible fat and yolk and pigment, which, indeed, may be driven to any part of the egg without prejudice to the eventual normality of development.

The evidence is, however, not altogether flawless. For, in the first place, we notice that in certain cases (Arbacia, the

Pulmonates) a return of the materials towards their original positions is described—a return which, moreover, appears certainly in the Pulmonates and possibly in the sea-urchin to be a condition of normal development as in the frog. In the second place, it does not follow that because the position of the egg-axis has not been hitherto affected by the centrifugal force employed it never can be so moved. Conklin, indeed, has succeeded in shifting it in Crepidula and Cynthia. The polar structure to which it belongs may therefore eventually prove to be dependent, to quote Conklin's own words, on "the heteropolar arrangement of certain oöplasmic substances," though these are indistinguishable to the eye, and need not, of course, be of sufficiently different specific gravities to allow the force applied to them to overcome their viscosities. When it is remembered that a solute can be centrifugally separated from its solvent, there is no need to despair of the possibility of still further separating from one another the materials of the egg cytoplasm.

Thirdly, the development of these embryos and larvæ has not always been followed either sufficiently closely or sufficiently long to vindicate the claim that is made for their normality.

In the present state of our knowledge, therefore, it is still possible that that polarity of egg-structure to which the development of the embryo is so intimately related is in these cases also itself dependent on a graduated concentration of different materials, and renewed investigation may justify the generalisation of this conception. That would probably enable us at once to express the polarity of the developing and the polarity of the regenerating organism in common terms. For the "gradation of materials," a formula originally proposed by Morgan himself, is certainly the best hypothesis yet put forward to explain one of the most constant features of regeneration—the formation of a regenerated structure with the same characters in the same direction as the original. Polarity may, of course, be reversed, but the reversal can usually be accounted for by supposing that the concentration

of a particular substance at a certain point has overcome the direction of gradation of the other substance (as, for example, when two Hydras are grafted together by their head ends, one cut off after the union is complete close to the graft plane, and a head instead of a foot regenerated from the cut surface).

Now though the polarity of an organism is often compared to the polarity of a crystal, experiment has made it abundantly clear that there is no real parallel between the two phenomena. Any fragment of a crystal has, of course, the same optical properties with the same orientation as the whole from which it was taken, no matter from what part of the larger piece it came; and any such fragment will regenerate the whole when suspended in the mother liquor. But with organisms it is not so. In certain cases (Protozoa, Hydroids, Planarians, some Oligochæts) any piece that is not too small, or at least any piece transversely cut out, will give rise to a complete organism, and the original polarity is observed in the process, but the proportions are not necessarily the same as in the original, and differ with the level or region of the whole from which the part was removed; and, indeed, the influence of region is frequently so great that in certain parts of the body the original structure cannot be replaced at all, as in the earthworm, where a head is never regenerated at an anterior cut surface behind a certain level, but a tail instead—another instance of region, or substance, overcoming polarity or the graduation of another substance. The hypothesis, of course, though the best available, remains a pure speculation, for the graduation of materials cannot be seen here as it can in the ovum.

It is not, however, only by means of the centrifuge that the causal relation between the polarity of the egg-cytoplasm, a certain arrangement whether of its visibly different or of its indistinguishable materials, and the formation of the organs of the embryo can be demonstrated. There is a whole series of experiments of another kind in which some

definite part of the ovum being removed, some definite organ or system of organs of the embryo is defective or lacking. Not only, therefore, does the polarity of the egg-cytoplasm determine the orientation of the parts of the embryo, but certain materials in it are causally associated with the eventual appearance of certain organs; the materials in question may, but need not be, situated in that region in which the organ will be developed, as in Dentalium, where factors on which the formation of both the anterior sense-organ and the trunk of the larva depend are both resident in the vegetative polar lobe of the egg.

In the cytoplasm, therefore, are placed, on both lines of evidence, the causes on which the primary differentiation of the embryo depends. Not that it is proved or even necessary to suppose that every separately inheritable organ or character of an organ is represented by some distinct material, for there is evidence that fresh structures may be developed by the interactions of already existing parts on one another. Given, indeed, some heterogeneity to start with, and the rest, however complex it may be, will follow in a regular and orderly sequence.

And this primary heterogeneity resides in the cytoplasm, which, therefore, is a vehicle of inheritable characters. The characters which the cytoplasm of the egg-cell thus transmits appear to be those which give the organism its rough outline, the large features which place it in its proper phylum, class, order, family, perhaps, which make it an Echinoderm and not a Vertebrate or a Mollusc, an Echinoid and not an Asteroid, and such general characters are probably transmitted only by the cytoplasm, and, therefore, only by the female cell, if we may trust the evidence of heteregeneous hybridisations. The smaller characters, of course—generic, specific, varietal—which might be more correctly described as so many modifications of the larger ones, are obviously transmissible equally by the germ-cells of the two sexes; that is, are carried by the chromosomes or smaller chromatic elements of their nuclei. But even the different chromatic

elements require a difference in the cytoplasm before they can exert each their appropriate activity, and call forth each the character to which each is beforehand appropriate. For every cell in the body contains in its nucleus a complete set—indeed, in sexually produced organisms two complete sets—of the chromatin elements or determinants characteristic of the species, since nuclear division is quantitative always, with the exception of the maturation division of the germ-cells. It is hard to conceive of the several chromatic determinants coming into operation, each at the right place and time, save in a heterogeneous medium. Given such a medium, then, this nuclear element will become active here, and that there, this will produce a specific modification of one character, that of another. And it is in the cytoplasm that this primitive and necessary heterogeneity is found, and from the cytoplasm that the prime factors of differentiation may, we hope, be isolated, and perhaps by means of the centrifuge.

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EXPLANATION OF PLATES 7–12,

Illustrating Dr. J. W. Jenkinson's paper "On the Relation between the Structure and the Development of the Centrifuged Egg of the Frog."

PLATE 7.

Fig. 1.—O. 1. *a*, from the equator; *b*, from the animal pole; *c*, segmentation.

Fig. 2.—O. 2. *a*, from the equator; *b*, from the animal pole; *c*, *d*, segmentation.

Fig. 3.—O. 3. *a*, from the animal pole; *b*, segmentation.